A Study of

Microbial Coal Desulphurisation

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PREFACE

The main objective of this study was to determine the capability of acidophilic microorganisms in removing sulphur from coal.
The study was divided into two parts as follows:
Part I dealt with inorganic (pyritic) sulphur removal from coal by using a mixed culture with *Thiobacillus ferrooxidans* dominant.
Part II was a study of organic sulphur removal from coal employing an isolated acidophilic culture.

Part I
Microbial inorganic (pyritic) sulphur removal:

In part I of this thesis, the capacity of a mixed culture, *Thiobacillus ferrooxidans* dominant, to remove inorganic (pyritic) sulphur is presented.
It begins with an introduction to microbial coal desulphurisation followed with a review of the chemical reactions to give an idea of the wonderful work of microorganisms in pyritic sulphur removal. Next, the objectives of the work are described.
In Chapter two, the background of experimental design such as oxygen requirement and transfer, reactor configurations and other experimental parameters i.e. slurry concentrations, stirrer speed and temperature are discussed. Next, the procedure to isolate microbial cultures, *Thiobacillus ferrooxidans* dominant from acid mine water is presented.
In Chapter three, results are presented together with a discussion of the effect of air supply, stirrer speed and temperature on microbial activity.
In this microbial process, pyritic sulphur is converted to an acid form, which can be detected by pH change of the reaction medium during processing. An equation relating pyritic sulphur removal and pH is proposed. This equation allows direct monitoring of the progress of coal desulphurisation. This is reviewed in Chapter four.
In Chapter five, the kinetic aspects of microbial coal desulphurisation are introduced. The kinetic rate equation is reviewed and tested.
The overall conclusions and recommendations are set out in Chapter six.

Copies of two papers submitted at the 18th Australasian Chemical Engineering Conference, 1990, and the Fourth New Zealand Coal Conference, 1991, are provided in the Appendices at the conclusion of the thesis.

Part II
Microbial organic sulphur removal:

Part II of the thesis deals with organic sulphur removal from coal using acidophilic cultures (designated HB1 and HB2) which were isolated from the effluent of the previous inorganic sulphur removal experiments.
This part of the thesis is divided into four chapters.
Chapter one is an introduction in which objectives of the study are presented together with a review of literature and current knowledge of microbial organic sulphur removal. This is followed by a consideration of some supporting factors and problems/limitations connected with the microbial organic sulphur removal from coal. Next, the structure of coal and its organic sulphur content are reviewed to give an idea of chemically bonded structure of the organic sulphur in the coal matrix. This has a bearing on the accessibility of the organic sulphur to microorganisms.
In Chapter two, isolation of the "new" acidophilic cultures (HB1 and HB2) which are capable of digesting organic sulphur from coal is described, followed by a preliminary identification of the isolated microorganisms.
In Chapter three, experimental results are presented. The relation of the rate of organic sulphur removal to particle size is explained together with the characteristics of the cultures in dealing with different coal types (pretreated, "fresh" Benneydale coal and New Creek coal). The effect of temperature on the rate of organic sulphur removal is also discussed. Next, the composition of the nutrient mediums is reviewed.
In Chapter four, recommendations for further work are proposed.

PART I

INORGANIC (PYRITIC) SULPHUR REMOVAL BY MICROBES

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PART I

INORGANIC (PYRITIC) SULPHUR REMOVAL BY MICROBES

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• *"Coal desulphurisation in stirred batch reactor using Thiobacillus ferrooxidans dominant, mixed bacterial cultures"*. In "Proceedings of Chemeca 90, The Eighteenth Australasian Chemical Engineering Conference, Processing Pasific Resources", volume II, August 27-30, 1990, Auckland, New Zealand, p. 1109-1115.

• *"The correlation of pyritic sulphur removal with pH values from coal in water slurries containing mixed acidophilic culture"*. In "Proceedings of the Fourth New Zealand Coal Conference",.14-16 October 1991, Wellington, New Zealand, p. 49-57.

ABSTRACT

A mixed microbial culture in which *Thiobacillus ferrooxidans* was predominant, isolated from acid mine water from the Stockton plateau, New Zealand has shown the capacity to remove pyritic sulphur from Benneydale coal.

Laboratory experiments using stirred batch reactors with a working volume of 1.3 litre indicated that most (92%-95%) of the pyritic sulphur in Benneydale coal had been removed after 12 days of processing. The performance of this culture was affected by several variables, i.e. coal slurry concentration, air flow rate, stirrer speed and temperature. Experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm showed a lower rate of pyritic sulphur removal, especially when conducted with a high slurry concentration (35% weight/volume). Increasing the air flow rate to 1.5 litre/minute significantly improved the rate of sulphur conversion. On the other hand, increasing the stirrer speed from 125 rpm to 175 rpm had little or no effect on the performance of this mixed culture.

By using the Van't Hoff temperature coefficient the optimum temperature for the mixed culture was found to be within the range of (35-40)^{o} Celsius, although the culture still endured at 45^{o} C.

During the process the pH of the medium changed from an initial pH of 2.6 to 1.4 at the end of the process. An equation relating pyritic sulphur removal and pH is proposed. This equation allowed direct monitoring of the progress of coal desulphurisation provided the pH of the medium was measured; this approach was easier and more precise than a process involving sulphur content analysis. The predicted data calculated using this pH equation was similar to the experimental data.

Kinetic studies of the microbial desulphurisation process showed that the reaction was approximately first order with respect to the substrate of pyritic sulphur concentration.

The activation energy E_a was found within the range of 8.8 - 14.2 (kcal/mole) for experiments using an air flow rate of 1.0 litre/minute

and stirrer speed of 125 rpm; within the range of 2.2 - 5.3 (kcal/mole) for the experiments at 1.5 litre/minute and 125 rpm; and within the range of 2.7 - 4.9 (kcal/mole) for the experiments at 1.5 litre/minute and 175 rpm.

CHAPTER 1

INTRODUCTION

1.1. GENERAL INTRODUCTION

Most coals contain sulphur which occurs in two dominant forms, namely, inorganic (pyritic) sulphur and organic sulphur which may be chemically bound to coal macromolecules or in the mobile phase. During the combustion of coal these sulphur compounds are mostly converted to sulphur oxides which have adverse effects on the biosphere and contribute to the acid rain problem. The removal of sulphur compounds, either before or after combustion, would significantly reduce environmental pollution. Various physical and chemical methods have been developed for desulphurisation of coal prior to combustion, see figure 1.1.

Desulphurisation of coal by microbes offers significant operating and environmental advantages over the physical/ mechanical and chemical methods (Kargi and Robinson, 1985). One disadvantage of microbial coal desulphurisation is that it generally takes longer and requires large reactor volumes and holding ponds.

The majority of microbial coal desulphurisation experiments have been carried out using shaken flasks. Detailed information on the rate controlling step for slurries in stirred reactors is scarce. Several parameters such as pH, air flow rate, stirrer speed and temperature affect the microbial desulphurisation of coal (Torma, 1977; Olson and Kelly, 1986).

An acidophilic mixed culture dominated by *Thiobacillus ferrooxidans* , isolated from acid mine water has been used in stirred batch reactors. Using a stirred batch reactor these parameters can be varied and controlled quite easily in order to observe their effects on the rate of pyritic sulphur removal.

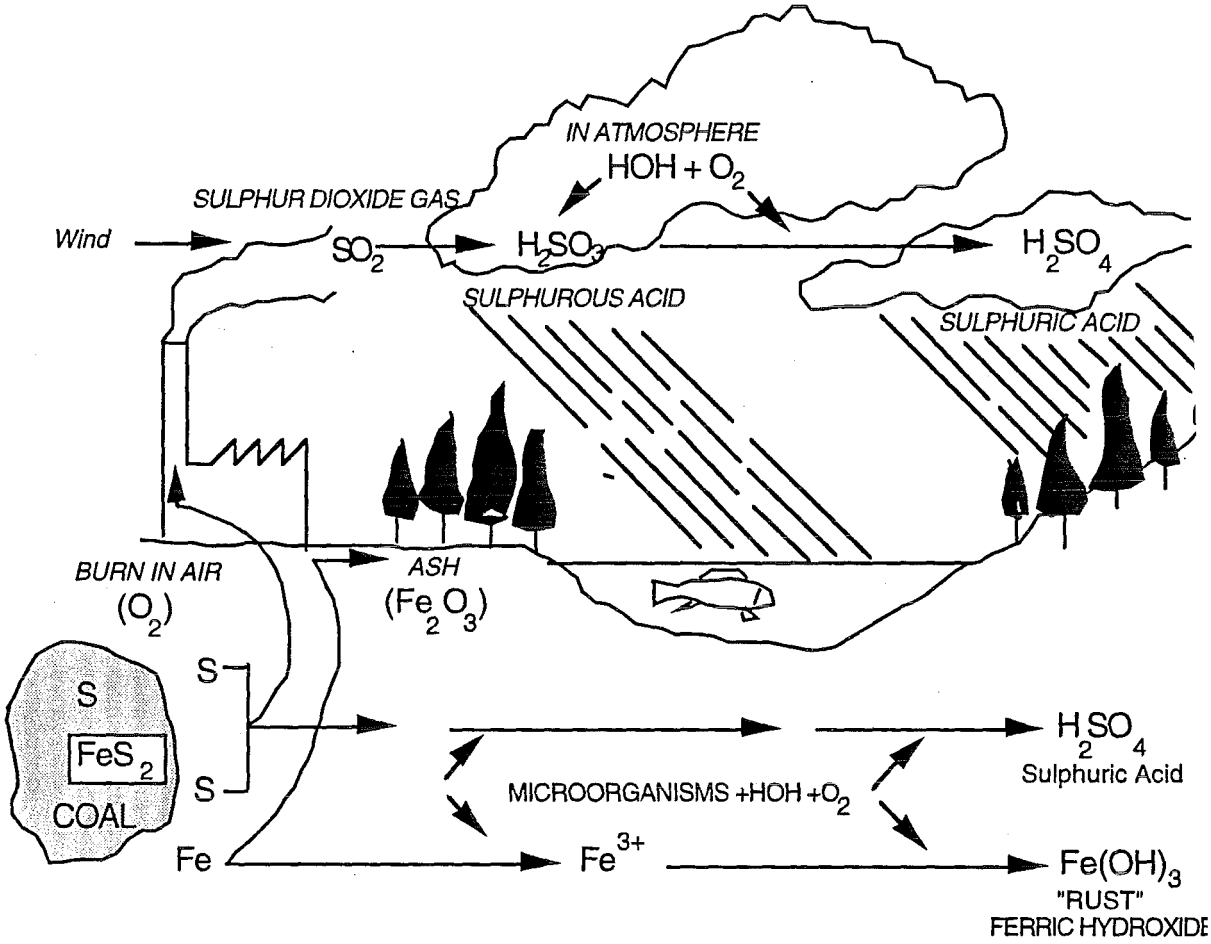


Figure 1. 1. Comparison of sulphur oxidation by combustion and by microbial metabolism.

The pH changes in the growth medium observed during an experiment are a consequence of microbial sulphur metabolism. The kinetic aspect has an important role for further application to microbial coal desulphurisation and understanding this part of the process is necessary (Olson and Kelly, 1986).

1.2. CHEMICAL REACTIONS DURING MICROBIAL COAL DESULPHURISATION

The pioneering work of Colmer and Hinkle in 1947 prompted numerous studies on the biological transformation of iron pyritic sulphur. These results suggested that mechanisms of these bioprocesses are either the direct attack of the pyritic sulphur by the bacteria or indirect dissolution of the pyritic sulphur via a bacterially generated oxidant (Brierly, 1978). Direct oxidation occurs via contact between the bacteria and the pyritic moiety of the coal whereby pyritic sulphur is oxidized to sulphate and Fe^{2+} to Fe^{3+}. Indirect oxidation can occur without bacterial intervention. Ferric ion which is produced by the bacteria's oxidative attacks on the ferrous ion in pyritic sulphur, chemically oxidizes the ferrous moiety of pyritic sulphur. The major role of bacteria in the indirect oxidation is to regenerate the ferric ion and this greatly accelerates the chemical oxidation of pyritic sulphur.

A scheme combining both direct and indirect reactions, as well as the physical structure of the coal (Hone *et al.* 1987) is shown in figure 1.2.

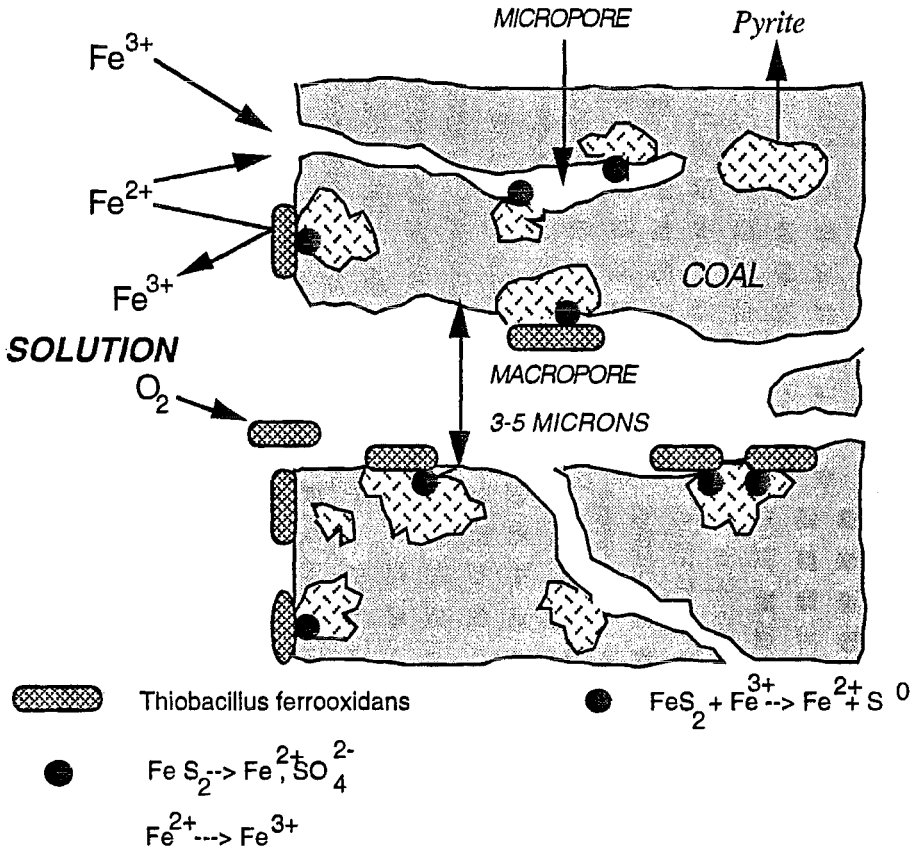


Figure 1. 2. Bimodel coal pore structure and oxidation of pyritic sulphur

Direct oxidation of pyritic sulphur by microorganisms occurs only at the outer surface of coal and within its macropores (3-5 μm), where direct contact between bacteria and pyritic sulphur is possible. Pyritic sulphur within the coal matrix can only be dissolved by chemical oxidation with ferric ion.
Since the bacteria are strict aerobes, oxygen is required regardless of whether the reactions occur via the indirect or direct oxidation.
In the case of iron pyritic sulphur, the overall oxidation, including direct and indirect oxidation is described by the equation (1.1).

$$4\ FeS_2 + 15\ O_2 + 2\ H_2O \text{ ----> } 2\ Fe_2(SO_4)_3 + 2\ H_2SO_4 \qquad (1.1)$$

This overall oxidation reaction represents the sum of a number of reactions which are occurring to yield the two main products. The initiating reaction where pyritic sulphur is exposed to oxygen and water, is a slow spontaneous reaction. It can be catalyzed by some bacteria, i.e.

$$2\ FeS_2 + 7\ O_2 + 2\ H_2O \text{ ---(bacteria)--> } 2\ FeSO_4 + 2\ H_2SO_4 \qquad (1.2)$$

The generation of hydrogen ions in this reaction leads to the development of acidic conditions, under which the auto-oxidation of ferrous ion is very slow.
In the presence of various bacteria, the best known of which is *Thiobacillus ferrooxidans* , the ferrous ion generated in this reaction can be rapidly oxidized. More specifically, Murr *et al.* (1978) suggested that this reaction was about 10 million times faster than the same reaction in the absence of bacteria.

$$4\ FeSO_4 + O_2 + 2\ H_2SO_4 \text{ -(bacteria)-> } 2\ Fe_2(SO_4)_3 + 2\ H_2O \qquad (1.3)$$

This reaction (equation 1.3) is the rate limiting step in the overall pyritic sulphur oxidation (and acidity consuming process). The ferric ions produced from this reaction (equation 1.3) can chemically degrade the pyritic sulphur by the following reaction:

$$FeS_2 + Fe_2(SO_4)_3 + 3\ O_2 + 2\ H_2O \text{ ---> } 3\ FeSO_4 + 2\ H_2SO_4 \qquad (1.4)$$

There is some evidence, that this reaction (equation 1.4) proceeds by two steps, one of which is bacterially catalyzed (equations 1.5 and 1.6).

$$FeS_2 + Fe_2(SO_4)_3 \text{ ---> } 3\ FeSO_4 + 2\ S \qquad (1.5)$$

$$2\ S + 3\ O_2 + 2\ H_2O \text{ -(bacteria)-> } 2\ H_2SO_4 \qquad (1.6)$$

Reaction 1.6 is important because it is believed to prevent a layer of elemental sulphur from accumulating on pyritic sulphur surfaces, inhibiting further reactions (Tributch and Benneth, 1981).
Some of the ferric ion produced may react to form insoluble hydroxides (ferric hydroxides) and more acid according to the reaction:

$$Fe_2(SO_4)_3 + 6\ H_2O \text{ ----> } 2\ Fe(OH)_3 + 3\ H_2SO_4 \qquad (1.7)$$

The pyritic sulphur oxidation cycle is illustrated in figure 1.3.

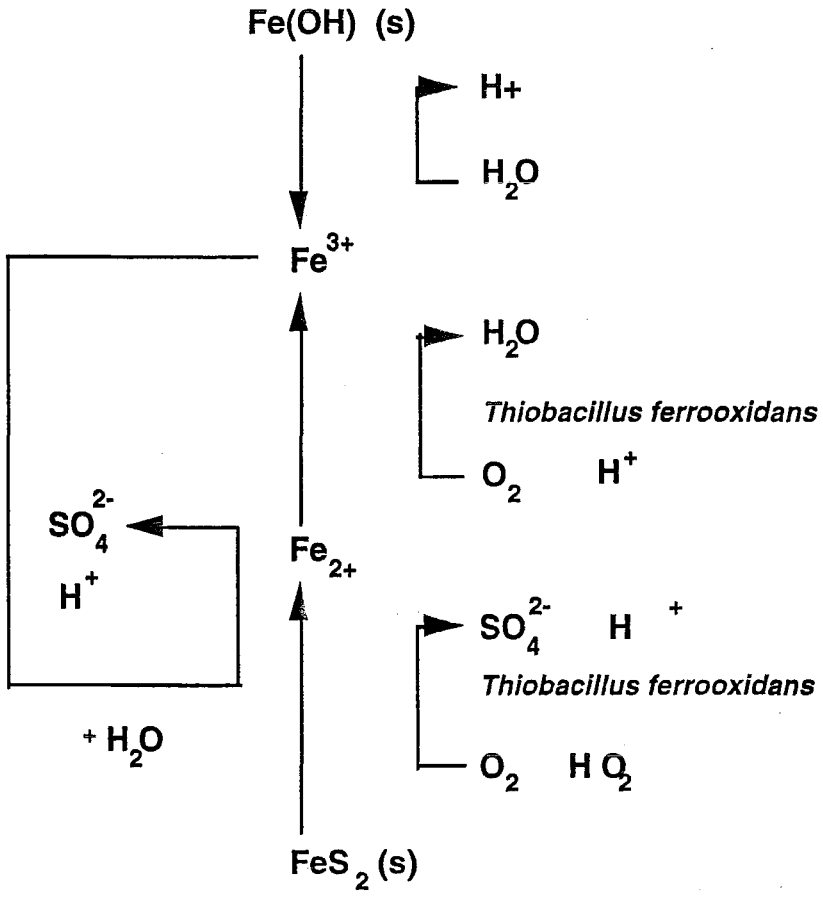


Figure 1. 3. The pyritic sulphur oxidation cycle and its relation with *Thiobacillus ferrooxidans.*

This figure shows the general reactions with products in the presence of *Thiobacillus ferrooxidans.*

1.3. THIOBACILLUS BACTERIAL CULTURES

The genus *Thiobacillus* is the best known of the sulphur oxidizing bacteria. Cells are gram negative, short rods. Most of the species are motile by means of a single polar flagellum. These bacteria are widely distributed in soil, fresh waters, coal mine drainage water (acid mine water) and marine environments. The most widely known is *Thiobacillus ferrooxidans.*

Thiobacillus ferrooxidans is a short rod, about (1-2) x 0.5 μm, occurring singly or in pairs, and occasionally motile. It is aerobic and does not form spores. It is acidophilic, growing optimally at pH 2-3 and at moderate (mesophilic) growth temperatures (20-35)°C. *Thiobacillus ferrooxidans* is chemoautotrophic; it oxidizes inorganic substances, viz. ferrous ions and reduced sulphur species, as primary energy sources. It obtains its cellular carbon needs from fixing CO_2 from air. It possesses the ability to oxidize reduced iron and sulphur as well as insoluble pyritic sulphur. It also oxidizes a wide variety of other metal sulphides. In these cases, energy is derived through sulphur oxidation, although reduced copper (Erlich, 1978), tin (Lewis and Mier, 1977) and uranium (DiSpirito and Tuovinen, 1982) may also serve as energy sources.
One question which is often raised when the commercial exploitation of these bacteria is considered concerns the possible effects of these metals on microbes. Although various metals are necessary in biological systems as trace elements, when the concentration exceeds certain low limits the metal may be toxic or at least inhibitory. The sulphur oxidizing bacteria can exist under a wide range of concentrations of various ions although they are sensitive to sudden changes in the solution composition of their environment. *Thiobacillus ferrooxidans* is a robust organism. It may be inhibited (but not killed) by low concentrations (50 ppm) of, for example copper and can be adapted over a period of time to tolerate much higher concentrations, of the order of 30,000 ppm. Bacterial tolerance to metal ions may be obtained by slowly increasing metal concentrations in culture medium. By slowly developing the

tolerance for specific metals, it has been possible to produce bacterial cultures active in solutions with high metal concentration

The other important species in this group is *Thiobacillus thiooxidans*. This organism is morphologically similar to *Thiobacillus ferrooxidans* and is often found in waste dumps and water together with *Thiobacillus ferrooxidans* . The fundamental difference between the two species is generally recognized to be the inability of *Thiobacillus thiooxidans* to oxidize ferrous ion and insoluble heavy metal sulphides. On the other hand this bacteria has the ability to utilize elemental sulphur for energy derivation.

1.4. AIMS AND OBJECTIVES OF THIS WORK

(i). To isolate suitable microorganisms from acid mine waters which could be used as an inoculum in laboratory experiments.
(ii). Monitoring the capacity of the isolated microbes to remove pyritic sulphur from coal in stirred batch reactors. Particular attention was paid to factors such as air flow, stirrer speed, and temperature.
(iii). To investigate the relationship of reactor pH values and the rates of pyritic sulphur removal and
(iv). to investigate the kinetics of sulphur removal by microbes in the reactor.

CHAPTER 2

EXPERIMENTAL

2.1. BACK GROUND

One of the important factors ensuring successful aerobic microbial processes is suitable aeration and mixing of microbial cultures (Brown, 1970; Tsao and Lee, 1977). The rate of oxygen consumption by aerobic microorganisms may be so high that even at maximum saturation of the medium with air the oxygen present may be consumed within 15 seconds (Sikyta, 1983). Thus, if oxygen supply is interrupted for a mere 15 seconds, the respiration of highly aerobic microorganism may be seriously impaired. Insufficient oxygen always causes reduced yields of microbial products such as organic acids, enzymes and reduced growth of the microorganisms. For practical purposes oxygen limitation is therefore undesirable and the oxygen concentration has to be kept up to ensure adequate availability to all cells (Hixson and Gaden, 1950).

There are two important criteria for maintenance of cell growth in aerobic processes:

- the maintenance of a dissolved oxygen concentration which is above the critical concentration required for exponential growth of the organisms (typical values ranging from 0.003 to 0.05 mM/l) (Jackson, 1990). Finn (1967) indicated that at dissolved oxygen concentration below 0.005 to 0.2 mM O_2/litre cell respiration diminished sharply.
- the supply of oxygen at a rate matching the metabolic utilization by the organism. In an aerated culture when a steady state is reached, the supply

of oxygen by dissolution into the liquid must equal the rate of demand by the organism for dissolved oxygen (Arnold and Steel, 1958).
Considerations of oxygen supply to microbial processes usually include simultaneous aeration and mixing since both processes affect the oxygen level. The experimental data from Bartholomew *et al.* (1950) showed clearly the necessity of using both aeration and agitation in aerobic microbial processes. The air flow rate and agitation speed can be controlled to maintain the desired dissolved oxygen concentration in the medium (Kargi and Moo-Young, 1985).

2.1.1. OXYGEN REQUIREMENT AND TRANSFER

Oxygen is sparingly soluble in water which severely restricts the rate of transfer from the gas phase to the liquid phase. The low solubility of oxygen is therefore an important factor when considering the continuous supply of oxygen to respiring cultures.
The transfer of oxygen from the gaseous phase proceeds by molecular diffusion. The double-film theory proposes the formation of a gaseous and liquid film at the gas-liquid interface; all the resistance to the transfer of oxygen from the gaseous into the liquid phase is then concentrated in the liquid film (figure 2.1).

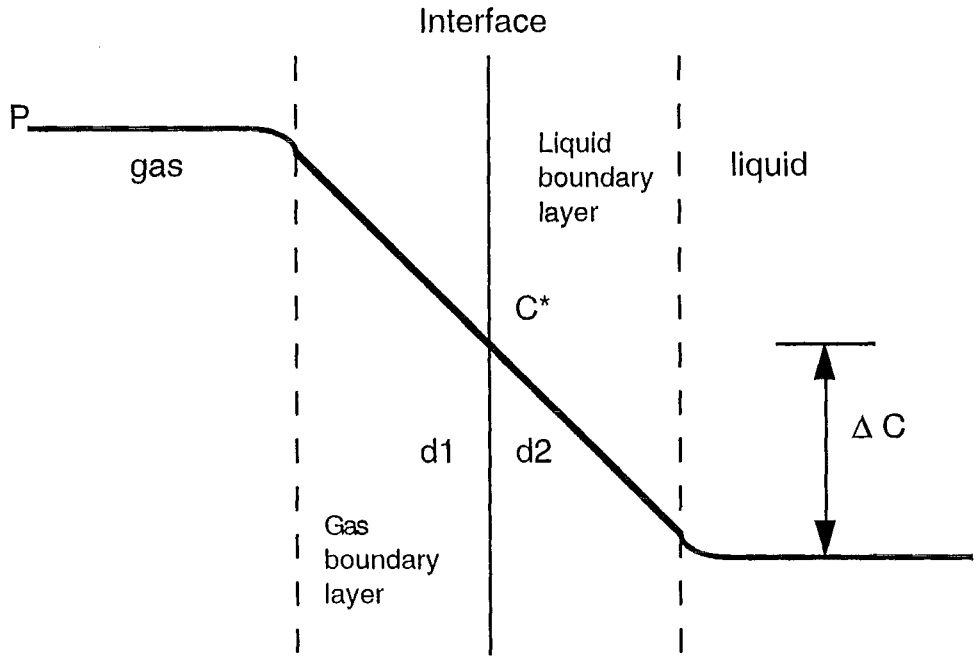


Figure 2. 1: Scheme of oxygen transfer from gas to liquid
d1 = film thickness

d2 = liquid film thickness (m)
P = partial pressure of diffusing gas (atm)
C* = equilibrium oxygen concentration (mol. m^{-3})/ (M. m^{-3})
C = concentration of dissolved oxygen (mol. m^{-3})
Δ C = driving force

If the oxygen concentration profile in the film is linear, no reaction occurs in the film and the absorption of oxygen is physical in nature. In the case of chemisorption the profile is not linear. The magnitude of resistance of the liquid film depends on the physico-chemical properties of the liquid and film thickness is affected by mixing intensity.
The double-film theory presumes an immediate equilibration between the oxygen concentration in the gas and in the liquid when the two phases come into contact.

$$P/He = C \qquad \text{Henry's law} \tag{2. 1}$$

The ratio of partial pressure / concentration is Henry's constant, where:
He = Henry's constant.

The solution of the diffusion process by the double-film theory is based on the first of Fick's laws

$$N = - D \, (dC/dx)_{x = d} \tag{2. 2}$$

The diffusion flow is proportional to the concentration gradient at any given distance, x, where:
N = diffusion flow (mol m^{-2} s^{-1})
D = oxygen diffusivity (m^2 s^{-1})
dC/dx = gradient
x = distance (m)

This equation yields:

$$N = (D/d) \, (C^* - C) \tag{2. 3}$$

$$N = k_L \, (C^* - C) \tag{2. 4}$$

where: k_L = coefficient of oxygen transfer (m s^{-1}).

When the rate of oxygen absorption is referred to the total surface area of suspension A in volume V_L,

$$V_L (dC/dt) = k_L A (C^* - C) \quad (2.5)$$

The mass transfer rate of oxygen per unit volume is then called the oxygen transfer rate,

$$(dC/dt) = k_La (C^* - C) \quad (2.6)$$

where: k_La = volume coefficient of oxygen transfer characterizing the aeration efficiency of the system (s^{-1}). Here a = specific interface area in mass transfer or gas-liquid interfacial area per unit volume (m^{-1}),
C^* = equilibrium oxygen concentration (mol. m^{-3}) ,
C = actual concentration of dissolved oxygen at a given moment.

In an aerobic biological system where the oxygen is consumed, the equation for oxygen transfer has the form (Sikyta, 1983):

$$dC/dt = k_La (C^* - C) - r X \quad (2.7)$$

where:
r = respiration rate of the microorganisms,
X = cell concentration.
Mixing has a positive effect on the oxygen transfer rate since it increases the value of k_La.
Uhl *et al* . 1989, successfully applied an air flow rate of 0.46 vvm (volume air flow per volume slurry per minute) in their coal desulphurisation experiments. Unfortunately, data of the critical oxygen concentration for *Thiobacillus ferrooxidans* is not available but based on the above it was deemed necessary to keep the concentration of oxygen in the slurry close to 0.2 mM O_2/litre. This was achieved by using an air flow rate of 1.0 litre/minute and 1.5 litre/minute which corresponds to 0.19 mM O_2 per litre and 0.28 mM O_2 per litre per minute (based on solubility of O_2 in water = 1.16 mM/l at 30°C and 20.99% volume of oxygen in air; see sample calculation) were continuously supplied to the reactor. Sample of

calculation: 1.0 l/minute of air = [(0.2099 x 1 x 1.16)/ 1.3] = 0. 187 mM/litre per minute of O_2 (volume of liquid in reactor = 1.3 litre). 1.0 litre/minute and 1.5 litre/minute corresponds to 0.77 vvm and 1.15 vvm, respectively.

2.1.2. SPEED OF STIRRER ROTATION

Besides the oxygen concentration in the liquid being sufficient it is necessary to ensure an adequate rate of oxygen transfer to the coal particles and microorganisms. Keeping the solids in suspension by stirring is an obvious way of achieving this.

Stirred slurry reactors are three-phase reactors in which gas and solid phase are dispersed in a continuous liquid phase. They are often used as laboratory reactors for process studies. One reason for their wide use is that they can accommodate stirrers with a variety of shapes permitting the reactors to fulfill many different process requirements.

One of the applications of rotating stirrers is the suspension of a solid in a liquid. In many cases, it will be sufficient if the liquid is well mixed and all the particles of the solid phase are in suspension, so that the whole solid surface is well exposed to the liquid. Suspension of the solid is necessary in slurry reactors in which mass transfer occurs between the liquid and solid phase.

Varying degrees of suspension are possible ranging from:

- complete suspension, in which the particles are just suspended and
- homogeneous suspension, in which the particle concentration is uniform throughout the reactor.

According to Zwietering (1958), in the state of complete suspension, no particle remains at rest on the bottom of the tank for longer than 1 or 2 seconds. The stirrer speed at which this state is attained, Njs, is called the minimum speed for complete suspension. At N < Njs, a portion of the solids remains in contact with the bottom of the reactor.

Njs depends on the settling velocity of the solids, reactor geometry, turbulence, and the physical properties of the liquid.

For liquid-solid systems, the minimum speed may be evaluated from an emperical expression suggested by Zwietering (1958):

$$\mathbf{Njs} = \mathbf{S} \cdot (\mathbf{d_p^{0.2}} \mu_l^{0.1} \mathbf{g^{0.45}} \Delta\xi^{0.45} \mathbf{B^{0.13}}) / (\xi_l^{0.55} \mathbf{D^{0.85}}) \qquad \textbf{(2. 8)}$$

where:

S = a constant depending on geometry, shape of the bottom, type of the stirrer, stirrer diameter (D), reactor diameter (T), and distance between the stirrer and the bottom of the reactor (C).
dp = particle size of the solid (m)
μ_l= liquid viscosity (Pa. s)
g = acceleration of gravity (m^2/s)
$\Delta\xi$ = difference in solid and liquid densities (kg/m^3)
ξ_l = liquid density (kg/m^3)
B = weight of the solids in suspension, per weight of liquid, times 100 (percent)
D = stirrer diameter (m)

A schematic drawing of the stirrer reactor used in this study is shown in figure 2. 2.

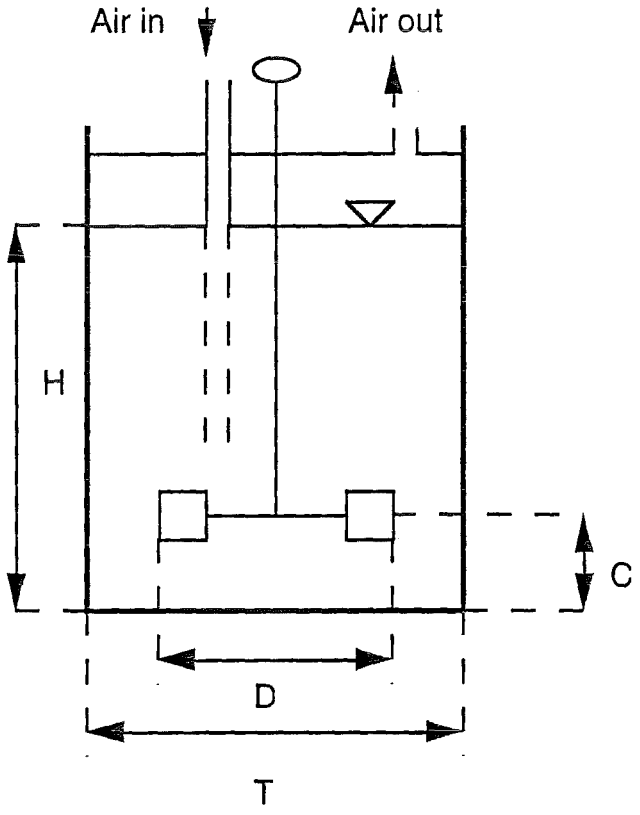


Figure 2. 2. : Stirred slurry reactor configuration
T = diameter reactor (= 0.12 m)
D = diameter stirrer (= 0.085 m)
C = distance between the stirrer and the bottom of the reactor (= 0.03 m)
H = height of the liquid (m; depending on slurry concentration).
Type of stirrer: four inclined blades

Minimum speed of stirrer

The reactor configurations and the other data used to calculate the minimum speed Njs can be seen below.
Data used to calculate the minimum speed (Njs) are as follows:

- dp= 152 E-6 m
- μl = 9.8 E-4 Pa s
- g = 9.81 m^2/s
- ξs = 1345.512 kg/m^3
- ξl = 1004.34 kg/m^3 (at 30°C)
- $\Delta\xi$ = 341.172 kg/m^3
- B = 15%; 20%; 25%; 30%; 35%
- T = 0.12 m
- D = 0.085 m
- C = 0.03 m
- S = Zwietering constant which is within the range (2.3 to 2.9) (for various types of stirrer; Zwietering, 1958).

The minimum speed Njs calculated based on Zwietering formula is shown in table 2.1 .

Table 2.1. Calculated minimum speed Njs

slurry conc. % w/v	Minimum speed Njs (rpm) s= 2.3	2.4	2.5	2.6	2.7	2.8	2.9
15%	124	130	135	140	146	151	157
20%	129	134	140	147	151	157	162
25%	133	149	145	151	157	162	168
30%	136	142	148	153	159	165	171
35%	140	146	153	159	165	171	177

The minimum speed Njs for the experimental conditions using the reactor with the configurations and parameters given above was estimated to be within the range (124-177) rpm.

- Using the above, it was decided to use only two stirrer speed rotations; 125 rpm and 175 rpm.

2.1.3. SLURRY CONCENTRATION

Kargi and Robinson (1982a) compared the amount of sulphur removal from an untreated coal slurry at several concentrations. They found that in an experiment with 5% of slurry concentration, 50% of the total (96% of the inorganic) sulphur was removed, while at 10% of slurry concentration, 40% of the total sulphur was removed. At higher slurry concentrations (15 to 30%), particle agglomeration was evident along with reduced solubility and transfer of oxygen. Detz and Barvinchak (1979) found that for slurry concentration greater than 20% by weight, the rate of desulphurisation decreased significantly. Several authors agreed that slurry concentrations up to 20-30% did not affect the desulphurisation (Olsen *et al.* 1980; Huber *et al.* 1984; Beyer *et al.* 1986a). A higher slurry concentration decreased the rate and large cell concentrations were also needed at high slurry concentration (Beyer *et al.* 1986b).

• It was therefore decided to use slurry concentrations from 15% to 35%w/v.

2.1.4. TEMPERATURE

All microorganisms have temperature optima-temperature ranges at which they function most efficiently. *Thiobacillus ferrooxidans* is mesophilic (optimum growth at moderate temperatures (20-35)^{o}C) and optimum temperature for microbial oxidation of pyritic sulphur by this microorganism is in the range (28-35)^{o}C (Detz and Barvinchak, 1979). Karaivko *et al.* 1977 indicated that the optimum temperature for microbial oxidation of pyritic sulphur lay within the range of (30-45)^{o}C and the biological oxidation ceased at around 55^{o}C and at higher temperatures only chemical oxidation occurred. In addition to the bacterial activity in direct pyrite oxidation, the rate of chemical pyrite oxidation also increased with rising temperature (Klein *et al.* 1988)

• Based on the above, the experiments in this study were conducted at temperatures within (30-45)^{o}C.

2.2. FURTHER EXPERIMENTAL DETAILS

2.2.1. COAL

Benneydale coal collected and advised by Coal Research Association of New Zealand, Inc., was used throughout this study. This coal contains pyritic sulphur (0.9-1.2)%. All coal samples used in experiments were ground to a particle size of < 152 μm.

The composition of Benneydale coal is presented in table 2.2.

Table 2.2.

The composition of Benneydale coal (#)

Composition	Coal no 44/302	Coal no 44/303
Total moisture	24.5%	22.0%
Moisture	18.5%	17.9%
Ash	6.4%	7.6%
Volatiles	36.8%	36.6%
Fixed carbon	38.3%	37.9%
Calorific value (MJ/kg)	22.50	22.37
Sulphur total	2.15%	2.32%
Forms of sulphur		
Sulphate	0.04%	0.03%
Pyritic	0.91%	1.24%
Organic	1.20%	1.05%

(#) Air dried basis at 70% R.H.

2.2.2. REACTOR AND EXPERIMENTAL CONDITIONS

Reactor:

The coal desulphurisation experiments were performed in two litre stirred batch reactors, each with a working volume of 1.3 litres (see figure 2.3); operating at equal temperatures, air flow rates and stirrer speeds but at different slurry concentrations.

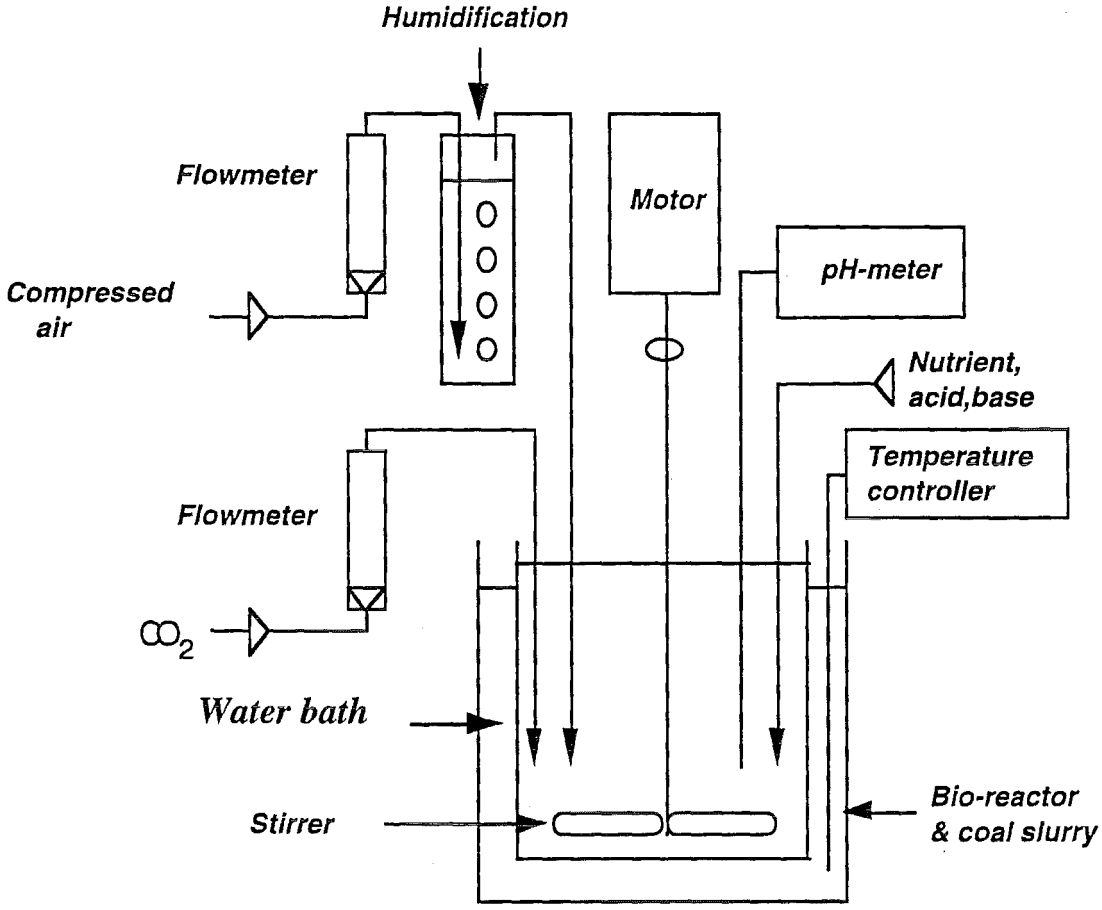


Figure 2. 3.: Schematic diagram of the experimental set up.

The reactors were charged with 1.2 litres mineral salts medium without $FeSO_4.7H_2O$ and coal particles of known particle size with slurry concentration in the range of 15% to 35%w/v.

The reactors were inoculated with 100 ml adapted cell liquid of *Thiobacillus ferrooxidans* .

All experimental work was conducted under non sterile conditions.

The initial pH of inoculated coal-salt medium mixtures was adjusted to pH 2.6 with 1.0 M H_2SO_4 and during the course of an experiment no attempt was made to maintain the pH at its initial value.

In this study the change of the medium pH was considered to be an important factor so its relationship to the rate of pyritic sulphur conversion was determined.

Experimental conditions:

- The experiments were conducted at 30°; 35°; 40° and 45° C.

- Stirrer speed: 125 and 175 revolution per minute (rpm).
- Air flow rate: 1.0 and 1.5 litre/minute.
- CO_2 flow rate: 5 ml per minute.

Each reactor was equipped with separate air and CO_2 lines to ensure the delivery of exact amounts of these gases.
To reduce evaporation of the liquid in the reactor, incoming gases were saturated by bubbling through water and any evaporation losses were compensated for by daily additions of distilled water.
Each experiment lasted for 24 days.

2.2.3. ANALYTICAL METHODS

- pH measurement:

A pH-meter, Radiometer, type PHM-26c, Copenhagen, Denmark, was used to measure the pH of the reaction mixture every day to monitor the rate of oxidation.

- Temperature:

Temperature was controlled automatically by using a waterbath (Grant Instruments Cambridge Ltd., Barrington, England) .

- Sulphur analyses:

Coal samples were withdrawn from the reactor every 6 days and obtained by filtration through Whatman filter paper no. 40. These coal samples were analyzed for sulphur content. For chemical analyses the coal samples were prepared and examined as follows:

- Total sulphur:

Standard ASTM D 4239, a high temperature tube furnace combustion method.

- Forms of inorganic sulphur (Pyritic and sulphate):

AS 1038, part 11, 1982.

- Organic sulphur:

The organic sulphur content of coal samples was determined indirectly from the difference between total sulphur and total inorganic sulphur (pyritic and sulphate) content of coal.
Sulphur determinations were carried out by Coal Research Association of New Zealand (Inc.), Gracefield, Lower Hutt, New Zealand.

Figure 2.4. shows the simplified flow chart of the coal desulphurisation process using the *Thiobacillus* mixed culture in this study.

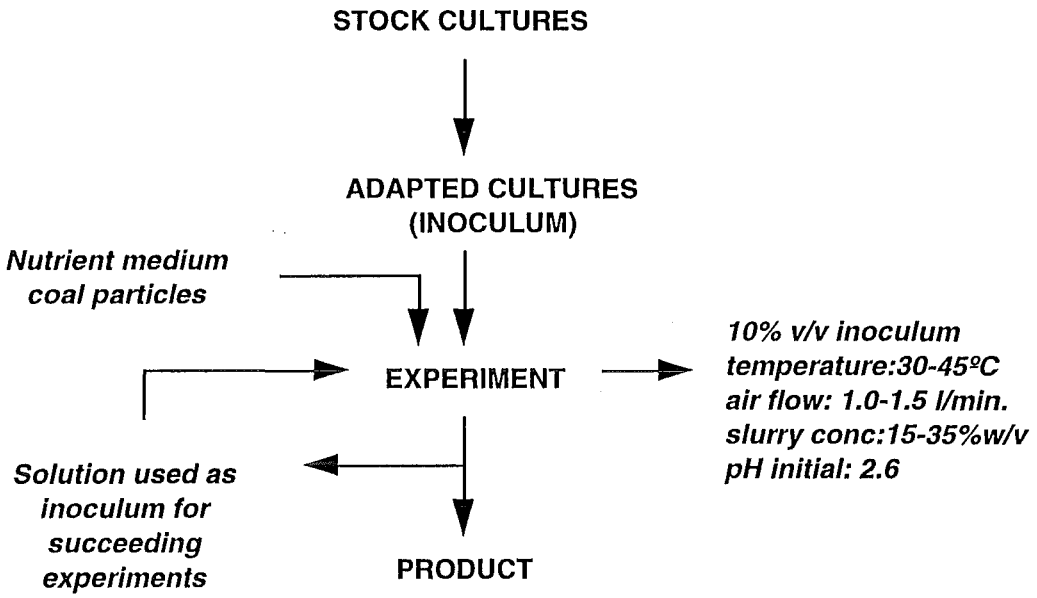


Figure 2. 4. Simplified flow chart of coal desulphurisation experiment

2.3. MICROBIOLOGICAL METHODS

The genus *Thiobacillus ferrooxidans* is the best known group in the category of sulphur compound metabolizing bacteria. *Thiobacillus ferrooxidans* is a mesophilic (20-45)^{o}C, gram negative, short rod like, acidophilic bacterium. It is aerobic and obtains carbon in the form of CO_2 from air. Its metabolic energy is derived from the oxidation of inorganic substrates such as ferrous ion and reduced sulphur compounds as well as insoluble pyritic sulphur.

Several investigations have studied pyritic sulphur degradation by mixed versus pure cultures of bacteria. Apel and Dugan (1972) found that a mixed natural population of acid mine drainage organisms increased the rate of pyritic sulphur oxidation. Kos *et al* . (1981) found that mixed cultures of acidophilic bacteria from the drainage of a coal washing plants were very effective in pyritic sulphur leaching from coal. In this study a mixed culture containing *Thiobacillus ferrooxidans* as the dominant microbe was used to convert the pyritic sulphur from coal. Hereafter this will be referred to as the *Thiobacillus* mixed culture.

The preparation of this inoculum for use in the present work is described in the next section.

2.3.1. ISOLATION OF THE CULTURE

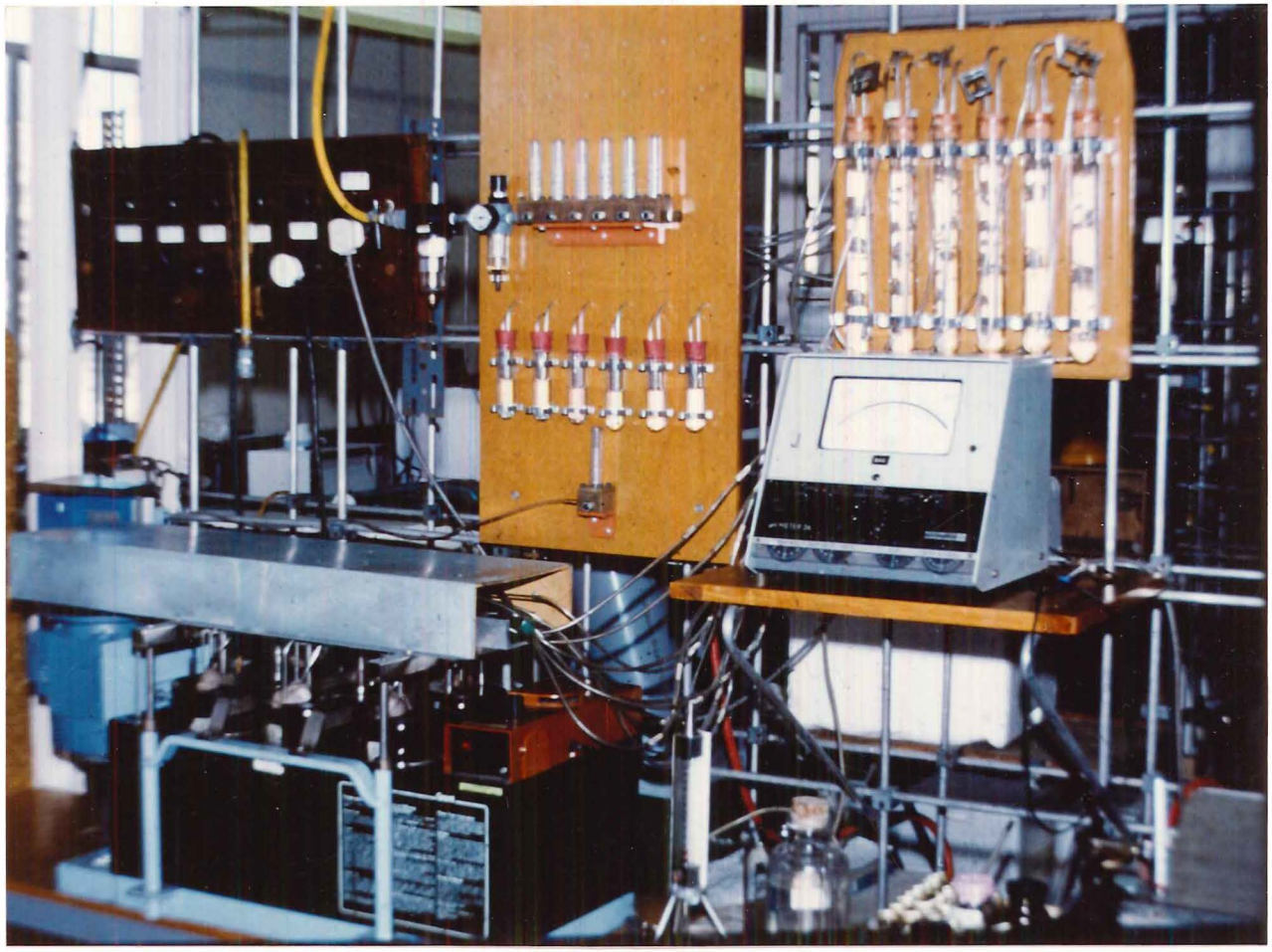
A strain of *Thiobacillus ferrooxidans* , isolated from acid mine water from Stockton Plateau, New Zealand was used throughout the experiments in this study.
The composition of the nutrient mineral salts medium used for growing the culture was:

$(NH_4)_2SO_4$ = 0.80 gram
KH_2PO_4 = 0.40 gram
$MgSO_4.7H_2O$= 0.16 gram
$FeSO_4.7H_2O$ = 20 gram
Distilled water = 1.0 litre

This was a formula recommended by American Type-Culture Collection, USA, for maintaining a pure culture of *Thiobacillus ferrooxidans*. The mineral salts were dissolved in sterilized distilled water and initially the pH of the medium was adjusted with 1.0 M H_2SO_4 to the desired pH. In this study, pH was initially adjusted to 2.6.
The organisms were cultivated in shake flasks in an incubator at a temperature of 30^{o} C.
Serial dilutions were made from the first culture produced. The cultures produced with this technique were the "stock" cultures.

2.3.2. ADAPTATION

In order to adapt the organisms to pyritic sulphur, which was to be the main energy source in the experiments, cells grown on the growth medium (stock cultures) were transfered to a nutrient medium with the same composition as in 2.3.1. but free of $FeSO_4.7H_2O$. In place of $FeSO_4.7H_2O$, coal particles which were ground and screened to < 152 μm were used. The amount of coal was 10% w/v.
By a dilution enrichment technique and after three transfers (with incubation times: 30; 21; and 21 days, respectively) in coal containing salt medium, cells were considered to be adapted to pyritic sulphur in coal. The adapted cultures were used as the inoculum for the first



Photograph 1: Experimental set-up

CHAPTER 3

EFFECT OF SLURRY CONCENTRATION, AIR FLOW RATE, STIRRER SPEED AND TEMPERATURE ON REMOVAL OF PYRITIC SULPHUR

3.1. INTRODUCTION

• Literature background

There have been numerous reports of bacterial coal desulphurisation in the literature and the bacteria involved in the oxidation of pyritic sulphur in coal almost invariably belong to the genus *Thiobacilli* (Silverman *et al.* 1961; Dugan and Apel, 1978; Detz and Barvinchak, 1979; and Hoffman *et al.* 1981).

Several parameters are reported to affect the rate and extent of microbial desulphurisation. Dugan and Apel (1978), Detz and Barvinchak (1979) and Hoffman *et al.* (1981) showed that slurry concentration of coal affected the rate of desulphurisation. Olsen *et al.* (1980) found that temperature was an important parameter and *Thiobacillus ferrooxidans* was reported to be metabolically active between 25 and 35°C. Huber *et al.* (1984) and Beyer *et al.* (1986a) showed that bacterial growth in stirred tank reactors was affected by the mixing behavior of a coal slurry (see also page 8). However, no information on the combined effects of these seems to exist in the literature.

3.2. RESULTS AND DISCUSSION

3.2.1. GENERAL OBSERVATION

The amount of the pyritic sulphur removal of the experiments are shown in table 3.1., table 3.2. and table 3.3 (raw data is presented in appendix B).

Table 3.1. represents the result of the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.
It was found that the amount of pyritic sulphur removal was within the range of:

(12.9 - 63.2)% on the sixth day,
(19.8 - 92.0)% on the twelfth day,
(52.6 - 96.0)% on the eighteenth day and
(67.2 - 100.0)% on the twenty-fourth day of the process.

Table 3.2. shows the amount of pyritic sulphur removal for the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm. The amount of pyritic sulphur removal was within the range of:

(36.2 - 83.5)% on the sixth day,
(68.5 - 95.3)% on the twelfth day,
(72.4 - 96.1)% on the eighteenth day and
(85.0 - 99.1)% on the twenty-fourth day of the process.

Table 3.3. shows the result of coal desulphurisation experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm .
The amount of pyritic sulphur removal was within the range of :

(10.7 - 66.4)% on the sixth day,
(37.9 - 94.3)% on the twelfth day,
(88.3 - 97.5)% on the eighteenth day,
(92.2 - 97.5)% on the twenty-fourth day of the process.

In each of the above sets of experiment, slurry concentrations of 15, 20, 25, 30 and 35% w/v were used.

Table 3.1

Pyritic sulphur removal for experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm

Temperature. (°C)	Coal slurry % w/v	% Pyritic sulphur removal at (day)			
		6th	12th	18th	24th
30	15	39.7	62.9	63.8	67.2
	20	36.2	52.6	73.3	74.1
	25	45.7	58.6	81.9	81.9
	30	12.9	43.1	79.3	83.6
	35	12.9	19.8	52.6	71.6
35	15	31.0	82.3	94.7	94.7
	20	48.7	77.9	92.9	93.8
	25*	0.0	n.a†.	10.6	18.1
	25	56.6	88.5	93.8	100.0
	30	60.2	86.7	92.0	94.7
	35	48.7	54.9	73.5	84.1
40	15	62.5	81.7	85.0	94.2
	20	56.7	80.0	83.3	90.8
	25	31.7	71.7	90.8	97.5
	30	41.7	75.8	91.7	95.8
	35	44.2	66.7	88.3	95.0
45	15	63.2	90.4	93.6	93.6
	20	50.4	62.4	63.2	83.2
	25	32.8	83.2	90.4	96.0
	30	47.2	92.0	96.0	98.4
	35	38.4	60.8	85.6	97.6

(*). Sterile medium without inoculum; (†). not analyzed

Table 3.2

Pyritic sulphur removal for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm

Temperature. (° C)	Coal slurry % w/v	% Pyritic sulphur removal at (day)			
		6th	12th	18th	24th
30	15	49.5	78.5	83.2	90.7
	20	50.5	80.4	83.2	89.7
	25	51.4	78.5	86.9	99.1
	30	37.4	77.6	92.5	93.5
	35	37.4	82.2	92.5	93.5
35	15	48.2	86.8	90.4	98.2
	20	37.7	84.2	90.4	95.6
	25	61.4	90.4	93.8	95.6
	30	43.9	86.8	95.6	95.6
	35	63.2	60.5	93.9	93.9
40	15	76.7	95.3	95.3	96.1
	20	68.2	90.7	90.7	92.2
	25	58.9	90.7	95.3	96.9
	30	58.9	90.7	94.6	96.1
	35	46.5	81.4	89.1	96.1
45	15	83.5	92.1	95.3	97.6
	20	36.2	68.5	72.4	85.0
	25	53.5	82.1	93.7	95.2
	30	57.5	94.5	96.1	97.6
	35	58.3	95.3	95.3	96.8

Table 3.3

Pyritic sulphur removal for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm

Temp. (° C)	Coal slurry % w/v	% Pyritic sulphur removal at (day)			
		6th	12th	18th	24th
30	15	35.0	82.5	90.3	93.2
	20	25.2	37.9	88.3	93.2
	25	26.2	64.1	93.2	96.1
	30	10.7	74.8	88.3	93.2
	35	17.5	72.8	91.3	92.2
35	15	47.4	88.8	92.2	95.7
	20	41.4	86.2	89.7	96.6
	25	42.2	85.3	92.2	92.2
	30	20.7	87.1	93.1	93.1
	35	24.1	78.4	88.8	94.0
40	15	66.4	87.6	93.4	94.2
	20	59.1	90.5	94.9	95.6
	25	59.9	88.3	92.7	95.6
	30	50.4	81.8	92.0	96.4
	35	38.0	81.0	94.2	96.4
45	15	58.2	94.3	96.7	97.5
	20	64.8	94.3	94.3	95.9
	25	62.3	84.4	91.8	94.3
	30	41.0	90.2	95.1	95.1
	35	40.2	92.6	97.5	97.5

These data show that almost all of the pyritic sulphur removal occurred within the first 12 days of the process although some experiments showed that it needed more time, around 18 days for total conversion. After that period the pyritic sulphur conversion changed only slightly, corresponding to a decline in microbial activity which is shown by the points after twelve days (see figure 3.1). This figure shows a typical graph of pyritic sulphur removal in one of the experiments (air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration of 25% w/v and temperature of 40°C).
The other experiments have similar profiles.

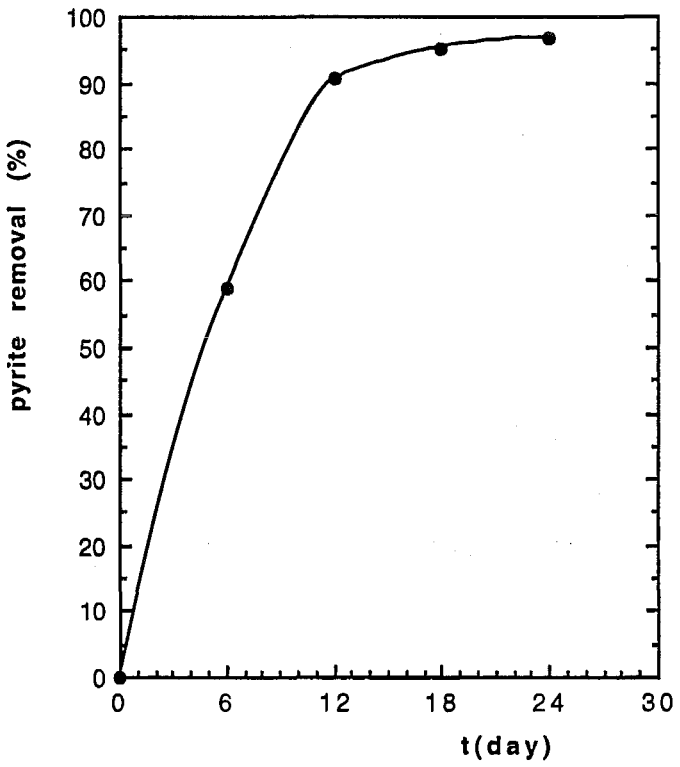


Figure 3. 1: Profile of pyritic sulphur removal for experiment conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration of 25% w/v and temperature of 40° C.

This profile can be explained in terms of the development of growth and stationary phases of microorganisms. According to Bailey and Ollis (1977), the nature of the growth phase is of potential importance in microbiological processes. The microbes used the pyritic sulphur available

in coal as their nutrient for growth and multiplication. This period (the active growth phase) occurred in the first 12 days following microbial inoculation and resulted in conversion of most of the pyritic sulphur during that time. During the stationary phase (> 12 days) the activity of the microorganisms in removing pyritic sulphur was very low and this resulted in less acid released during that time.

This result is consistent with the result of other studies. For instance, Hoffman *et al.* (1981) mentioned that a strain of *Thiobacillus ferrooxidans* isolated from a natural system was more effective and resulted in high levels of desulphurisation; (90-98)% in coal samples with a high pyritic sulphur content in the time frame 8 to 12 days. Dugan and Apel (1978) also found that 97 % of pyritic sulphur could be removed by leaching -200 mesh (-75 μm) coal at pH 2.0 to 2.5 in a process performed over a period of 5 days. They used a mixed culture, containing only *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans.*

Some results of other investigators are shown in table 3.4.

Table 3.4.

Other investigator's results (#).

Initial Pyritic sulphur content (%)	1.9	10.5	3.6	0.6-2.5	1.0
Particle size (μm)	< 74	147-417	< 500	< 100	< 500
Pulp Density (% w/w)	20	3.3	10	up to 20	10
Temp (° C)	28	28	30		45
Pyritic sulphur removal Max/durat (%/days)	90/16		90/14	80-90/9	80/14
References	Detz and Barvinchak (1979)	Kargi (1981)	Beyer *et al.* (1986b)	Huber *et al.* (1983)	Hone *et al.* (1987)

(#) Conditions may vary slightly concerning pH, nutrients and incubation systems, all experiments with pure or enrichment cultures containing predominantly *Thiobacillus ferrooxidans.*

After the microbes are introduced to the reactor, they will adjust and adapt to their new environment. This phase is known as the lag phase.
Figure 3.2. shows a typical growth curve of a microbial culture. The lag phase will be different for each type of microbe and their ability to adapt is influenced by many factors. It can last for several hours or even several days or months depend on their ability and environment factors such as nutrient, temperature, strain of the microorganisms, pH etc. The lag phase will be followed by exponential growth where the microbes grow rapidly resulting in fast rates of conversion of pyritic sulphur.

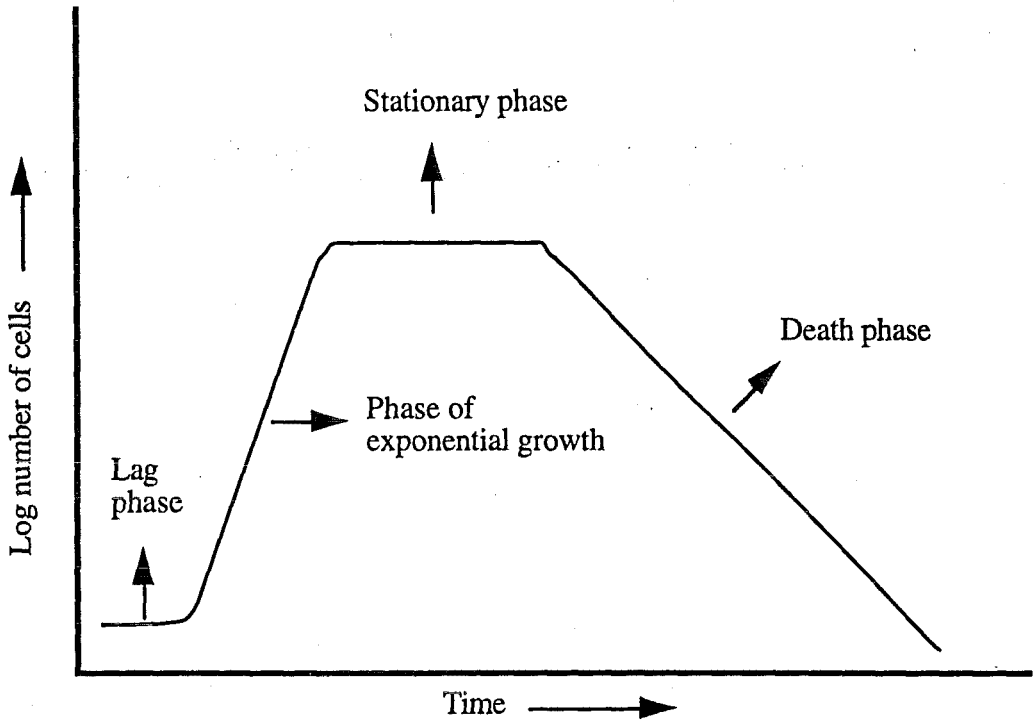


Figure 3. 2.: Typical batch growth curve of a microbial culture (Murphy *et al.* 1985).

In the profile of pyritic sulphur removal versus time as shown in figure 3.1, the lag phase can not be distinguished because there was no coal sampling done (coal sampling started at the sixth day). However, pH monitoring showed that the pH of the medium did not change during the first day of the process; moreover some experiments showed that the change of pH occurred 2 days after inoculation (see appendix C). It can be interpreted that the lag phase for this *Thiobacillus* mixed culture was within a period of (1-2) days after inoculation. During this lag phase the activity of the microorganisms in converting the pyritic sulphur was very

low corresponding to low pyritic sulphur removal and/or no acid released during that time. After this time, they grew exponentially causing a rapid conversion of the pyritic sulphur as indicated by the experimental data.

3.2.2. EFFECT OF SLURRY CONCENTRATION, AIR FLOW AND STIRRER SPEED

In this section, the effect of slurry concentration, air flow rate and stirrer speed on the rate of pyritic sulphur removal is discussed. The result of the experiments so far show that most of the pyritic sulphur in Benneydale coal had been removed in the first 12 days of the process. There is good reason to suppose that this period represents the actual time for the process to be completed. Consequently, 12 days was chosen as a benchmark time for analysis of results, i.e., the amount of sulphur removed after 12 days was used to compare the different experimental conditions.

(i). Effect of slurry concentration

Experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm

The amount of pyritic sulphur removal in the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm at different slurry concentrations (15-35%w/v) on day 12 of the process is shown in table 3.5.

Table 3.5.
Pyritic sulphur removal on day 12 of the process for experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.

t (°C)	Pyritic sulphur removal (%) for slurry concentrations				
	15%	20%	25%	30 %	35%
30	62.9	52.6	58.6	43.1	19.8
35	82.3	77.9	88.5	86.7	54.9
40	81.7	80.0	71.7	75.8	66.7
45	90.4	62.4	83.2	92.0	60.8

It shows that the experiments with 35% of slurry concentration resulted in the lowest amount of pyritic sulphur removal compared with the other experiments with slurry concentrations within the range 15-30% w/v. The low rate of pyritic sulphur removal obtained in the experiments with high slurry concentration (35% w/v) leads to an interpretation that concentrations above 30% may be rate inhibiting. This is supported by Beyer *et al.* (1986b) who mentioned that a higher coal content in microbial coal desulphurisation decreased the reaction rate. Several authors such as Detz and Barvinchak (1979); Olsen *et al* . (1980); Kargi and Robinson (1982a, b); Huber *et al.* (1984); and Beyer *et al.* (1986a) agreed that slurry concentration up to 20%-30% did not affect the desulphurisation.

In this study the lower rate of desulphurisation for slurry concentration 35% w/v (at air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm) was possibly restricted because of the limitation of dissolved oxygen and hence reduced mobility of the microbes. The movement of suspended coal in the reactor was not enough to maintain the ideal conditions for microbial action. The complicated flow patterns have a considerable effect on the interaction between the bacteria and their chemical reactions which resulted in a low rate of pyritic sulphur removal. Moreover, high slurry concentration means that there is more pyritic sulphur available in the system. Consequently a greater microbial population is required to digest the available food. *Thiobacillus ferrooxidans* is an aerobic microbe which needs oxygen to survive. Oxygen is the primary electron acceptor for iron and sulphur oxidation by *Thiobacillus* , so that oxygen must be available to the organisms for oxidation. In a process with high slurry concentration more microbes will demand more oxygen to support good growth and/or high rates of product synthesis. For aerobic processes the active cell concentration depends on the oxygen transfer which is the rate limiting step (Kleinstreuer and Poweigha, 1984):

$$\mu X = k_L a\,(C^* - C).\,Y_{O2} \qquad (3.1)$$

where:

Y_{O2} = the oxygen yield for growth and product formation

μ = specific growth rate

k_La = volume coefficient of oxygen transfer
C^* = equilibrium oxygen concentration
C = actual concentration of dissolved oxygen at a given moment.
X = cell concentration

The specific growth rate μ can be related to the rate of oxygen consumption Q_{O2} (Kargi and Moo-Young, 1985):

$$Q_{O2} = \mu / Y_{O2} \qquad (3.2)$$

At steady state, the oxygen transfer and consumption rates are equal, that is:

$$k_La\,(C^* - C) = \mu X / Y_{O2} = Q_{O2} X \qquad (3.3)$$

The rate of oxygen transfer is maximum when C = 0 and the maximum rate of oxygen consumption is achieved when $\mu = \mu_{max}$. If $k_La\ C^*$ is smaller than $\mu_{max} X / Y_{O2}$, then oxygen consumption is limited by the rate of oxygen transfer. When the reverse inequality holds, the reaction is limited by the microbial consumption of oxygen. If the oxygen supply is limited this situation is unfavourable for microbiological and chemical reactions resulting in lower productivity which in this study was indicated by low pyritic sulphur removal and less sulphuric acid release. If oxygen starvation at higher slurry concentration is considered to be the main factor causing lower rate of pyritic sulphur removal, then increasing k_La to improve culture oxygenation should overcome this problem. This can be done by increasing aeration and agitation rates (Olson and Kelly, 1986). Improvement of the conditions in this manner should improve the microbes' capacity to remove the pyritic sulphur.

To investigate this possibility other experiments with an increase of air supply (1.5 litre/minute instead of 1.0 litre/minute) were conducted (the other parameters were as same as before).

(ii). Effect of air flow rate

Experiments using an air flow rate of 1.0 and 1.5 litre/minute and stirrer speed of 125 rpm.

Experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm indicated that coal desulphurisation with higher slurry concentration (35% w/v) resulted in a lower pyritic sulphur removal and a slurry concentration of 30% w/v appeared to be the upper limit. Assuming that the reduced activity is due to lack of oxygen, increasing the air supply from 1.0 litre/minute to 1.5 litre/minute should lead to an improvement.

Data from the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm support this hypothesis. From table 3.6 it is evident that increase of air supply from 1.0 litre/minute to 1.5 litre/minute significantly improved the process. The amount of pyritic sulphur removal in these experiments was higher than the results of the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm which showed the benefit of increasing of air supply.

Table 3.6.

Pyritic sulphur removal on day 12 of the process for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

t (°C)	Pyritic sulphur removal (%) for slurry concentrations				
	15%	20%	25%	30 %	35%
30	78.5	80.4	78.5	77.6	82.2
35	86.8	84.2	90.4	86.8	60.5
40	95.3	90.7	90.7	90.7	81.4
45	92.1	68.5	82.1	94.5	95.3

As expected, most improvement was observed in the experiments with high slurry concentration 35% w/v. This improvement can be clearly seen in figure 3.3 which compares the minimum and maximum values of the amount of pyritic sulphur removal obtained in experiments conducted at air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm and 1.5 litre/minute and 175 rpm within temperature range 30° to 45°C with slurry concentration of 35% w/v.

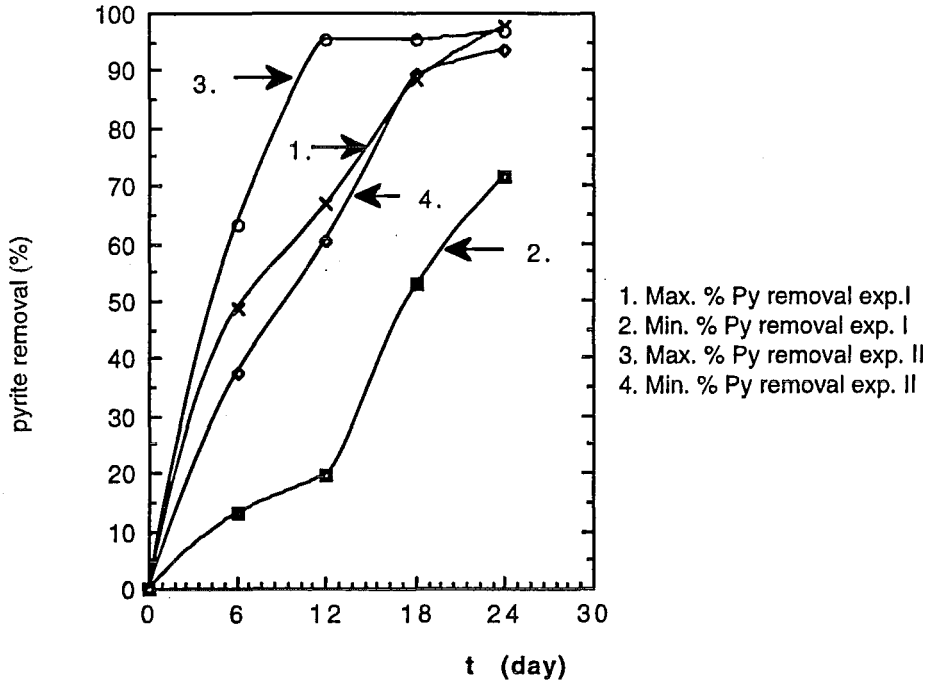


Figure 3. 3 : Comparison graph showing maximum and minimum % pyritic sulphur removal for experiment I and II. Experiment conditions:
I. Air flow rate of 1.0 litre/minute, stirrer speed of 125 rpm, 35% w/v.
II. Air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, 35% w/v.
Temperature: (30-45)^{o}C.

It can be seen that the maximum and minimum values of the amount of pyritic sulphur removal has changed significantly. The minimum line increases as does the upper limit which indicates an improvement due to the higher air supply to the reactor.
This observation shows an obvious advantage of increasing the air supply from 1.0 litre/minute to 1.5 litre/minute while the other variables such as stirrer speed, sparger type were kept constant. It was clear that for coal desulphurisation with high slurry concentration (35%), oxygen transfer becomes one of the rate limiting factors. This is supported by Bos *et al.* (1986) who mentioned that gas mass transfer becomes the rate limiting step in a coal desulphurisation process at high slurry concentration.
According to Arnold and Steel (1958) there were many other variables such as stirrer speed; type of stirrer; height of liquid in the reactor; type of sparger; and air velocity that affect oxygen supply. These are likely to

have a direct influence on the efficiency of the process. Due to time constraints, the influence of these variables was not investigated in this study.

(iii). Effect of stirrer speed

Experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

In the two experiments described above the other variable, stirrer speed, was maintained constant at 125 rpm. A stirrer speed of 125 rpm appeared to give adequate agitation for such experiments in this reactor. It is likely that an increase in the speed of rotation will have little or no effect on the rate of pyritic sulphur removal. To test this possibility the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm were conducted. The result can be seen in table 3.7.

Table 3.7.
Pyritic sulphur removal on day 12 of the process for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

t (°C)	Pyritic sulphur removal (%) for slurry concentrations				
	15%	20%	25%	30 %	35%
30	82.5	37.9	64.1	74.8	72.8
35	88.8	86.2	85.3	87.1	78.4
40	87.6	90.5	88.3	81.8	81.0
45	94.3	94.3	84.4	90.2	92.6

The comparison of the data of pyritic sulphur removal in table 3.6 and table 3.7 indicates that there are increases and decreases in the amount of the pyritic sulphur removal occurring during the experiments. Decreases in the amount of pyritic sulphur removal was observed in most experiments except in those cases using a slurry concentration 15% w/v at 35°C; 20%w/v at 35°C and 45°C; 25% w/v at 45°C; and 35% w/v at 35°C.

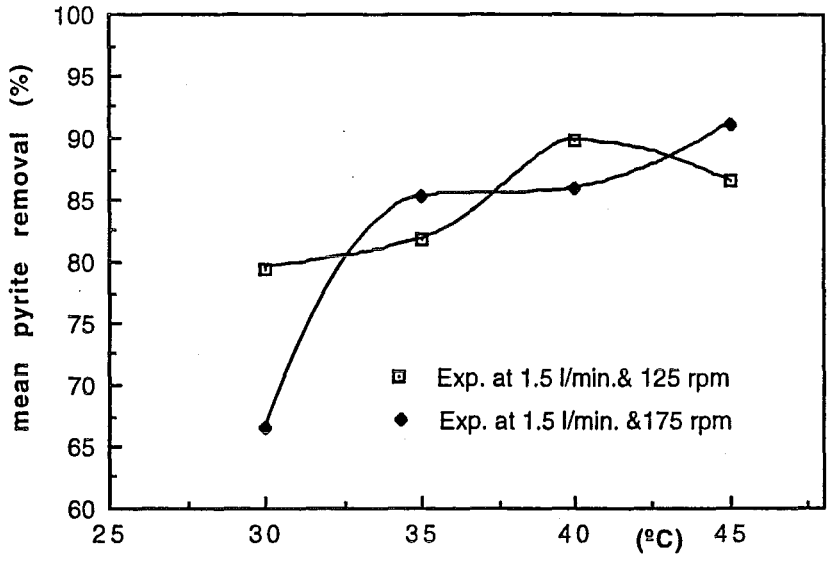


Figure 3. 4: Comparison of the mean values of pyritic sulphur removal in experiments with stirrer speed of 125 rpm and 175 rpm.

The mean values of the amounts of pyritic sulphur removal observed in experiments with stirrer speed of 125 rpm and 175 rpm are compared in figure 3.4. The results indicate that there is no clear advantage due to an increase of stirrer speed from 125 rpm to 175 rpm while maintaining air flow rate constant at 1.5 litre/minute.
It was concluded that stirrer speed of 125 rpm was sufficient for maintaining the coal desulphurisation process in this reactor. The effect of agitation could be studied further but it appears that there is no advantage in increasing the degree of agitation beyond the "just suspended" condition.

3.2.3. EFFECT OF TEMPERATURE

In these experiments the amount of pyritic sulphur removal at 30°C was generally less than that occurring at higher temperatures (35°, 40° and 45°C). This work showed that temperature is an important rate limiting factor and should be considered in a coal desulphurisation process. The effect of temperature on the rate of pyritic sulphur conversion in a coal desulphurisation process will be discussed.

To examine the effect of different temperatures on the rate of coal desulphurisation a quantitative treatment based on the Van't Hoff temperature coefficient can be made (Trumbore, 1966 and Voznaya, 1981). The mathematical expression for the temperature dependence of reaction rate is:

$$Q = \left(\frac{v_2}{v_1}\right)^{\frac{10}{(T_2-T_1)}} \qquad (3.4)$$

where

v_1 is the initial reaction rate at temperature T_1

v_2 is the reaction rate after a rise in temperature to T_2

Q is temperature coefficient of the reaction.

The reaction rate which increases with rising temperature is caused by an increase in the velocity of molecules and the frequency of collisions.
As mentioned by Voznaya (1981) the Van't Hoff temperature coefficient is only suitable for tentative calculation within a small temperature range because the coefficient itself changes with temperature.
It is derived as an approximation from the Arrhenius equation reaction rate, k = exp. (- E /RT), where E is the activation energy; R is the gas constant; and T is the absolute temperature.

These coal biodesulphurisation experiments were conducted at temperatures within the range 30°C to 45°C. This temperature range was chosen because the literature indicated that the optimum temperature for microbial oxidation of pyritic sulphur lies within this range (Karaivko *et al.* 1977). To examine the effect of temperature on the rate of coal desulphurisation the Van't Hoff coefficient (Q) for the rate of pyritic sulphur conversion has been calculated. This calculation was derived for every 5 degree Celsius increase of temperature.

The results of experiments concerning Q values for pyritic sulphur conversion within the range of temperature 30°C to 45°C at different air flow rate and stirrer speed are presented in tables 3.8; 3.9 and 3.10. They show that the temperature range between 35°C to 40°C was found to be the most effective, especially for those experiments using higher slurry concentrations; 30% and 35% w/v.

Table 3.8

Q values calculated from the amount of pyritic sulphur converted in experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm

Slurry concentration. % w/v	Day of process	Q values Q30/35	Q35/40	Q40/45
15	6	0.58	4.59	0.85
	12	1.62	1.11	1.33
	18	2.09	0.91	1.32
	24	1.88	1.12	1.07
20	6	1.71	1.53	0.86
	12	2.08	1.19	0.52
	18	1.53	0.91	0.62
	24	1.52	1.06	0.91
25	6	1.46	0.35	1.16
	12	2.16	0.74	1.46
	18	1.24	1.06	1.07
	24	1.41	1.07	1.05
30	6	20.55	0.54	1.39
	12	3.84	0.86	1.60
	18	1.28	1.12	1.19
	24	1.22	1.16	1.14
35	6	13.44	0.93	0.82
	12	7.27	1.66	0.90
	18	1.85	1.63	1.02
	24	1.31	1.44	1.15

In these tables:

- Q30/35 represents the Van't Hoff coefficient if temperature increases from 30° to 35°C;
- Q35/40 is the coefficient for temperature 35° to 40°C and
- Q40/45 is the coefficient if temperature increases further to 45°C.

According to the formula a Q value greater than one (> 1) indicates an improvement of the rate and a higher Q value means a better rate of desulphurisation.

Table 3.8. shows the Q values from the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.
The Q values on day 12 of the process are summarized in figure 3.5.

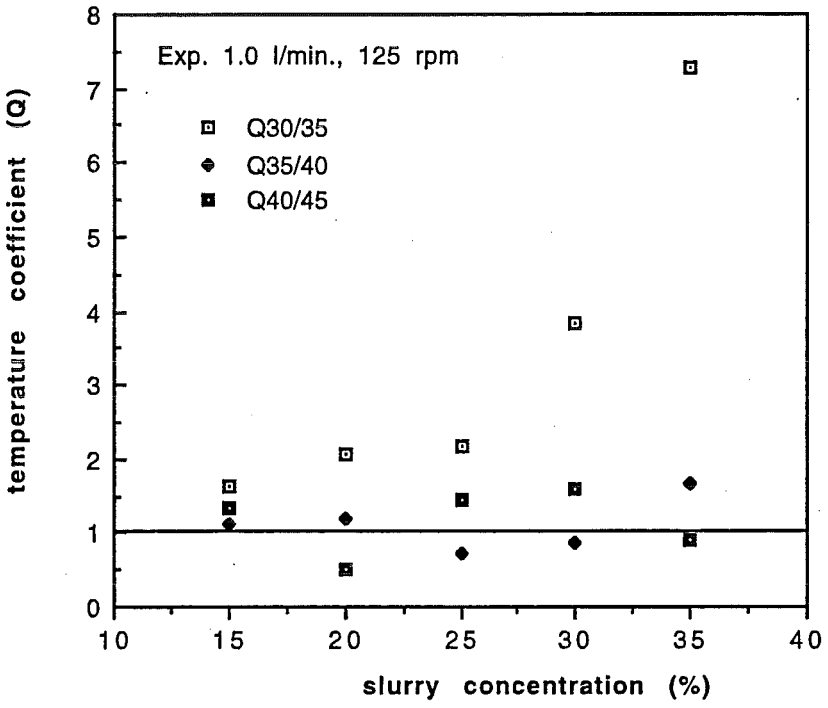


Figure 3. 5: Q values on day 12 of the process for experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.

This figure shows that increase of temperature from 30° to 35°C resulted in an increase in pyritic sulphur removal as shown by the Q30/35 values. Further increase of temperature from 35°C to 40°C resulted in a diminished performance of the microorganisms. All Q35/40 values are lower than Q30/35. A further increase of temperature from 40°C to 45°C produced a similar result which indicated little benefit in increasing temperature to 45°C.

Table 3.9

Q values calculated from the amount of pyritic sulphur converted in experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm

Slurry concentration. % w/v	Day of process	Q values Q30/35	Q35/40	Q40/45
15	6	1.08	3.24	1.15
	12	1.39	1.54	0.90
	18	1.34	1.43	0.97
	24	1.33	1.23	1.00
20	6	0.63	4.19	0.27
	12	1.25	1.49	0.55
	18	1.34	1.29	0.62
	24	1.29	1.19	0.82
25	6	3.22	1.18	0.80
	12	1.40	1.29	1.00
	18	1.19	1.32	0.94
	24	1.21	1.32	0.94
30	6	1.56	2.07	1.03
	12	1.42	1.40	1.05
	18	1.21	1.25	1.00
	24	1.19	1.29	1.00
35	6	1.10	2.04	1.52
	12	0.61	2.32	1.33
	18	1.17	1.16	1.11
	24	1.14	1.34	0.98

Overall, it was concluded that the most suitable experimental temperature was 35°C. This conclusion is supported by Torma (1986) who mentioned the optimum temperature for *Thiobacillus ferrooxidans* was around 35°C and that higher temperatures led to a lower rate of desulphurisation. Although the microbes are still active at 45°C, raising the temperature further will eventually cause immobilization and destruction.

Table 3.9. shows the Q values for the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm. Figure 3.6. represents the Q values observed on day 12 of the process for these experiments.

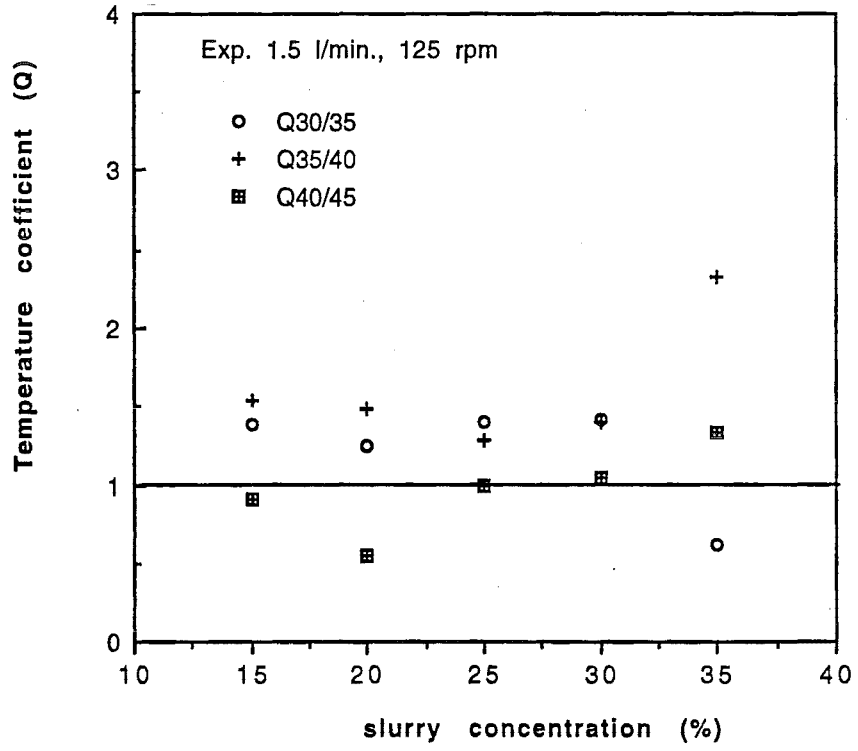


Figure 3. 6: Q values on day 12 of the process for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

In this figure, the data show that increasing the temperature from 30°C to 35°C and then from 35°C to 40°C resulted in an increase in pyritic sulphur removal as shown by the Q30/35 and Q35/40 values except for Q30/35 with 35% w/v of slurry concentration. Increase of temperature from 40°C to 45°C resulted in a low rate as shown by the Q40/45 values, except for the run with 35% w/v of slurry concentration. These patterns indicated that temperatures within the range (35-40)°C seem to be the

most suitable. A temperature of 40°C was observed to produce a slightly better result compared with 35°C.

Table 3.10. shows the Q values from the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.
The result is summarized in figure 3.7.

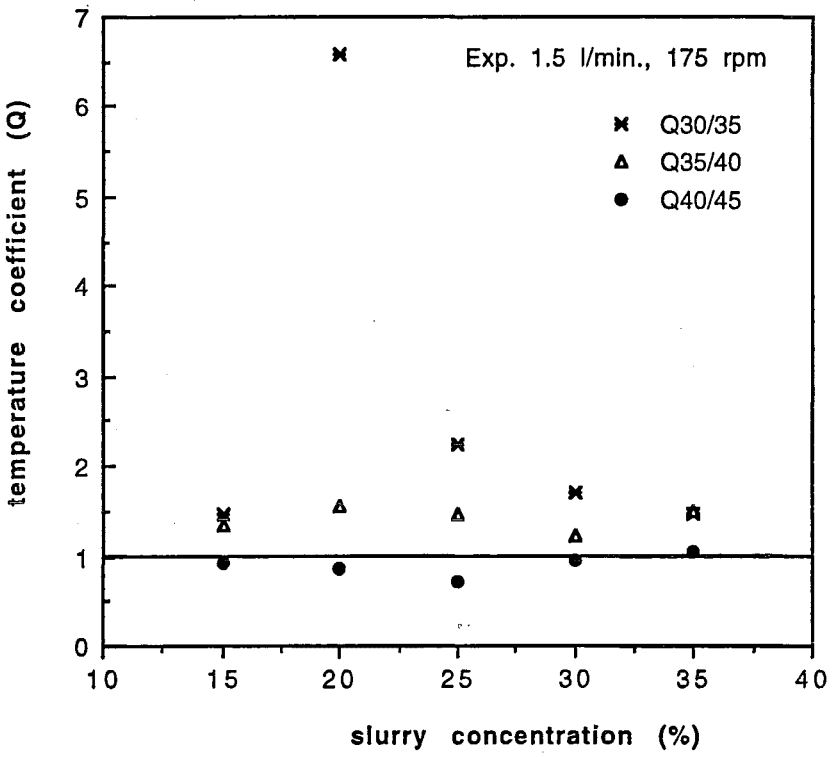


Figure 3. 7: Q values on day 12 of the process for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

In these experiments a similar trend was observed for Q30/35 and Q35/40. Increasing temperature from 30°C to 35°C and from 35°C to 40°C showed an increase in sulphur removal by microbes but not at 40°C to 45°C. The ability of the microorganisms to convert the pyritic sulphur decreased between 40°C to 45°C. These temperatures may have reduced the viability of microbes.

Table 3.10

Q values calculated from the amount of pyritic sulphur converted in experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm

Slurry concentration. % w/v	Day of process	Q values Q30/35	Q35/40	Q40/45
15	6	2.33	2.74	0.61
	12	1.47	1.36	0.92
	18	1.32	1.43	0.85
	24	1.34	1.35	0.85
20	6	3.41	2.85	0.95
	12	6.57	1.55	0.86
	18	1.31	1.56	0.78
	24	1.36	1.37	0.80
25	6	3.29	2.89	0.86
	12	2.25	1.48	0.72
	18	1.24	1.41	0.78
	24	1.17	1.50	0.77
30	6	4.76	6.04	0.72
	12	1.72	1.23	0.96
	18	1.41	1.36	0.85
	24	1.27	1.49	0.77
35	6	2.42	3.45	0.89
	12	1.47	1.49	1.04
	18	1.20	1.57	0.85
	24	1.32	1.47	0.81

The temperature of the process affects both the solubility (i.e. equilibrium dissolved oxygen concentration) and the diffusivity (i.e. transfer coefficient) of oxygen. The solubility of oxygen in water decreases with temperature (see table 3.11), but the diffusivity of O_2 in the liquid phase increases with absolute temperature (Kargi and Moo-Young, 1985).

Table 3.11.

Solubility of O2 at 1 atm in water at various temperatures.(*)

T ^{o} C	Water O2 mM/l
0	2.18
10	1.70
15	1.54
20	1.38
25	1.26
30	1.16
35	1.09
40	1.03

(*) Data from " International critical tables," Vol. III, p. 271, McGraw- Hill Book Company, N.Y., 1928.

The net effect of temperature on the rate of oxygen transfer depends on the range of temperatures considered. At low temperature ($10^{o}C<T<40^{o}C$) the increase in temperature is more likely to increase the rate of oxygen transfer due to an increase in the diffusivity of oxygen. However, at high temperatures ($40^{o}C<T<90^{o}C$), the solubility of oxygen drops significantly, which adversely affects the rate of oxygen transfer. Olson and Brickman (1986) also mentioned that less bioaction could be expected at a higher temperature due to the compounding effect of exothermic heat generated from the biological and chemical reactions.

3.2. CONCLUSION

The results of the experiments indicated that most of the pyritic sulphur (92-95%) in Benneydale coal could be removed after a 12 day processing period using a *Thiobacillus* mixed culture. The performance of this culture was affected by several variables, such as coal slurry concentration, air flow rate, stirrer speed and temperature. Experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm yielded a relatively lower rate of pyritic sulphur removal. An increase of air supply to the reactor improved the performance of the microorganisms significantly especially for the experiments with high slurry concentration (35% w/v), as shown by the result of experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

On the other hand, increasing the stirrer speed from 125 rpm to 175 rpm has little or no effect on the performance of this mixed culture. The stirrer speed of 125 rpm was sufficient for maintaining the performance of the coal desulphurisation process in the reactor with the configurations as used in this study. However, further studies of gas mass transfer and agitation rates are essential especially when high volumetric productivity is important.

The interpretation of Van't Hoff temperature coefficient (Q) values in this study for pyritic sulphur conversion lead to the conclusion that pyritic sulphur conversion can be enhanced by increasing temperature. It was found that the optimum temperature was in the range 35°C and 40°C. One investigator, Torma (1986) found that the optimum temperature for pure *Thiobacillus ferrooxidans* was 35° C. This difference is not surprising as the present coal desulphurisation experiments used a different strain of *Thiobacillus* in mixed microbial culture. In these experiments a strain of *Thiobacillus ferrooxidans,* isolated from acid mine water from Stockton plateau was used and no attempt was made to isolate a pure *Thiobacillus* strain which led to the possibility that there were other microbes which participated in the reactions. Particular bacterial strains and the nature of the coal as well as associated mineralogical inclusions could influence the rate of pyritic sulphur removal.

CHAPTER 4

THE RELATION OF pH CHANGE WITH PYRITIC SULPHUR CONVERSION

4.1. CHANGE OF pH VALUE

4.1.1. BACKGROUND

In a microbial coal desulphurisation system the microorganisms employed in the process will convert pyritic sulphur in coal to other forms according to the reaction:

$$2\,FeS_2 + 7.5\,O_2 + H_2O \text{ -----> } Fe_2(SO_4)_3 + H_2SO_4 \qquad (4.1)$$

The *Thiobacillus* mixed culture used in this investigation did the same thing and produced acid, which was detected using a pH-meter. The pH of the nutrient mixture in all cases was initially adjusted to 2.6 using 1.0 M H_2SO_4. This initial pH value was selected because it has been found to be optimal for *Thiobacillus* strains (Dugan and Apel, 1978 and Torma, 1986). Each experiment lasted 24 days during which there was no control or adjustment of the reaction mixture pH.

Tuovinen (1986) found that the lowest pH limit for *Thiobacillus ferrooxidans* was not well defined and microbial leaching experiments indicated that the bacteria was still active at pH values as low as 1.2 although long lag periods of several months were observed before microbial activity became apparent. According to Bruynesteyn (1986) at pH 1.6 problems due of cell stress were not evident and this pH usually

prevented the formation of insoluble Fe(III) complexes in leach liquor. In practice, for control of iron precipitation, pH must be less than 3.0.

4.1.2. RESULTS AND DISCUSSION

Tables 4.1 - 4.3 show the pH values observed on day 6, 12, 18 and 24 of experiments with different conditions of :
(a) air flow rate of 1.0 litre/minute and 1.5 litre/minute and
(b) stirrer speed of 125 rpm and 175 rpm.
The complete pH data from day 1 to 24 for all experiments are given in appendix C.

Table 4.1. shows the pH development during the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.
It was found that pH values decreased from the initial pH of 2.6 to the following:

(1.94 - 2.59) at day 6,
(1.48 - 2.06) at day 12,
(1.43 - 1.92) at day 18 and
(1.29 - 1.88) after day 24 of the desulphurisation process for slurry concentration of 15% to 35% w/v and temperature range 30^{o} to 45^{o} C.

Table 4.2 shows the results for the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.
pH of the slurry was within the range:

(1.74 - 2.43) at day 6,
(1.42 - 2.06) at day 12,
(1.36 - 1.94) at day 18 and
(1.33 - 1.89) at day 24 of the experiment.

Table 4.3 shows the results of the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm. The pH for these experiments ranged from:

(1.73 - 2.49) at day 6,
(1.44 - 2.10) at day 12,
(1.39 - 1.95) at day 18 and
(1.37 - 1.90) at day 24 of the process.

Table 4.1:

pH development for experiments with an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.

Temperature (° C)	coal slurry % w/v	pH value at day 6th	12th	18th	24th
30	15	2.49	2.06	1.87	1.82
	20	2.44	1.98	1.87	1.82
	25	2.41	1.92	1.87	1.82
	30	2.37	2.00	1.87	1.82
	35	2.39	1.89	1.87	1.82
35	15	2.37	1.96	1.83	1.72
	20	2.22	1.92	1.80	1.68
	25	2.03	1.82	1.68	1.57
	30	2.06	1.78	1.70	1.62
	35	2.13	1.92	1.78	1.68
40	15	2.38	1.88	1.72	1.54
	20	2.37	1.78	1.55	1.46
	25	2.29	1.76	1.48	1.36
	30	2.32	1.78	1.43	1.29
	35	2.37	1.74	1.48	1.33
45	15	2.15	1.68	1.66	1.59
	20	2.30	1.98	1.92	1.88
	25	2.11	1.57	1.49	1.46
	30	1.94	1.48	1.44	1.40
	35	2.03	1.53	1.43	1.36

Table 4.2:

pH development for experiments with an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

Temperature (° C)	coal slurry % w/v	pH value at day 6th	12th	18th	24th
30	15	2.43	2.06	1.94	1.89
	20	2.40	1.98	1.86	1.79
	25	2.36	1.92	1.78	1.73
	30	2.34	1.82	1.73	1.69
	35	2.26	1.81	1.73	1.63
35	15	2.28	1.87	1.80	1.77
	20	2.19	1.80	1.70	1.67
	25	2.04	1.69	1.62	1.58
	30	2.12	1.69	1.60	1.56
	35	2.36	1.89	1.65	1.59
40	15	1.95	1.79	1.70	1.67
	20	2.03	1.72	1.66	1.63
	25	1.98	1.66	1.57	1.51
	30	1.84	1.61	1.51	1.46
	35	1.93	1.61	1.52	1.48
45	15	1.79	1.61	1.56	1.55
	20	2.13	1.82	1.76	1.75
	25	1.83	1.50	1.44	1.42
	30	1.74	1.42	1.36	1.34
	35	1.85	1.44	1.38	1.33

Table 4.3:

pH development for experiments with an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

Temperature (° C)	coal slurry % w/v	pH value at day 6th	12th	18th	24th
30	15	2.49	2.10	1.95	1.90
	20	2.44	1.96	1.83	1.79
	25	2.41	1.93	1.78	1.74
	30	2.37	1.86	1.73	1.68
	35	2.39	1.80	1.69	1.64
35	15	1.95	1.74	1.64	1.62
	20	2.30	1.84	1.79	1.79
	25	2.21	1.74	1.66	1.66
	30	2.07	1.70	1.61	1.60
	35	2.20	1.67	1.59	1.57
40	15	2.06	1.71	1.68	1.65
	20	1.97	1.69	1.58	1.53
	25	1.92	1.62	1.57	1.50
	30	1.86	1.53	1.51	1.45
	35	1.87	1.52	1.47	1.42
45	15	1.84	1.61	1.58	1.55
	20	1.88	1.55	1.48	1.48
	25	1.73	1.51	1.43	1.38
	30	1.87	1.50	1.41	1.37
	35	1.95	1.44	1.39	1.37

An evaluation of these pH values suggests that microbial activity was optimum during the first 12 days of the process. High microbial activity during that period was evidenced by rapid conversion of the pyritic sulphur (see also chapter 3, page 27). This period was the exponential growth phase which was characterized by rapid formation of acid as shown by a rapid decrease in the pH of the reaction medium. This period was followed by a stationary phase during which the reproduction and death rates were in balance. In this phase the microbes died because the nutrients or energy sources essential for their production were exhausted (Murphy *et al*. 1985). During this phase reaction rate slowed and less acid produced.

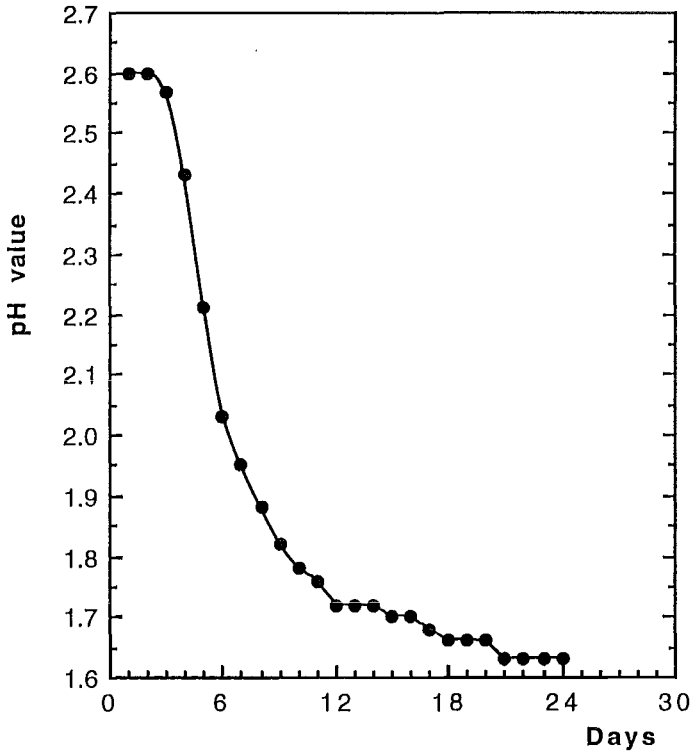


Figure 4. 1: Profile of pH values for an experiment conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration of 20% w/v and temperature of 40° C.

Figure 4.1 shows an example plot of changing pH versus time. The pH curve shows a rapid decline in the first 12 days of the process followed by a plateau. The plateau of the curve corresponds to a diminished net production of acid in the stationary phase during which there is a balance between the death and production of new microbes. In this phase,

nutrients or energy sources are no longer available for microbial multiplication.

Observation of the pH values on day 12 of the process indicated that the pH values obtained in experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm were lower than those observed in the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm. These data support the previous finding that an increase of air flow rate from 1.0 litre/minute to 1.5 litre/minute improved the process (page 34). On the other hand, comparison of the pH values obtained in experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm with those in experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm showed there were no significant difference which confirmed the previous conclusion that increase of stirrer speed from 125 rpm to 175 rpm had no clear effect on the process (page 36).

4.2. ESTIMATION OF PYRITIC SULPHUR REMOVAL BASED ON pH VALUE

In this study, calculation of pyritic sulphur conversion was based on standard analysis of sulphur content in coal samples before and after desulphurisation. Sulphur analysis is laborious, time consuming and therefore expensive.
In order to obtain more immediate information about the process, a suitable indicator which could be measured on line was sought.

4.2.1. LITERATURE REVIEW

Microbial coal desulphurisation studies in the main, have monitored either dissolved total iron or sulphate to calculate removal of pyritic sulphur from coal. Only one of these two substances was usually measured and decomposition was assumed to proceed stoichiometrically according to the reaction:

$$2\ FeS_2 + 7\ 5O_2 + H_2O \rightarrow Fe_2(SO_4)_3 + H_2SO_4$$

Several publications dealing with this subject have appeared. For example, Kargi (1982) calculated pyritic sulphur removal using the stoichiometric relationship between the sulphur and iron content of pyritic sulphur (i.e. S pyritic sulphur/Fe pyritic sulphur = 1.143). On the other hand, Andrews and Maczuga (1982) measured both iron and sulphate content, because they found that if only iron release was measured a misleading picture of coal desulphurisation rate was obtained.
Limited information is available on the use of other methods to calculate the overall removal of the pyritic sulphur from coal. The possibility of the use of other methods is reported by Huber *et al.* (1983). They compared the different analytical methods e.g. total iron, sulphate and oxygen consumption available. They showed the suitability of the microbial oxygen consumption rate based on gas phase analysis as a measure for pyritic sulphur oxidation.
Other researchers, e.g. Hone *et al.* (1987) employed the redox potential to calculate the pyritic sulphur conversion with a Pachuca tank reactor. In their experiments, examining pyritic sulphur conversion by *Thiobacillus*

ferrooxidans , an increase in the ferric ion concentration of the medium caused an increase in the redox potential. The redox potential varied directly with the conversion of pyritic sulphur.
Klein *et al.* (1988) mentioned that data based on the analysis of the sulphur content before and after desulphurisation were scarce due to the laborious and time consuming nature of sulphur testing by standard analytical methods.

4.2.2. RESULTS AND DISCUSSION

Relation between pH and pyritic sulphur concentration

As shown in the previous chapter the concentration-time plots of pyritic sulphur in the coal phase and pH of the medium versus time showed that increasing amounts of pyritic sulphur removal caused increasing acidity of the reaction medium. Based on this, it can be argued that the pH change represents the extent of the pyritic sulphur oxidation or vice-versa.

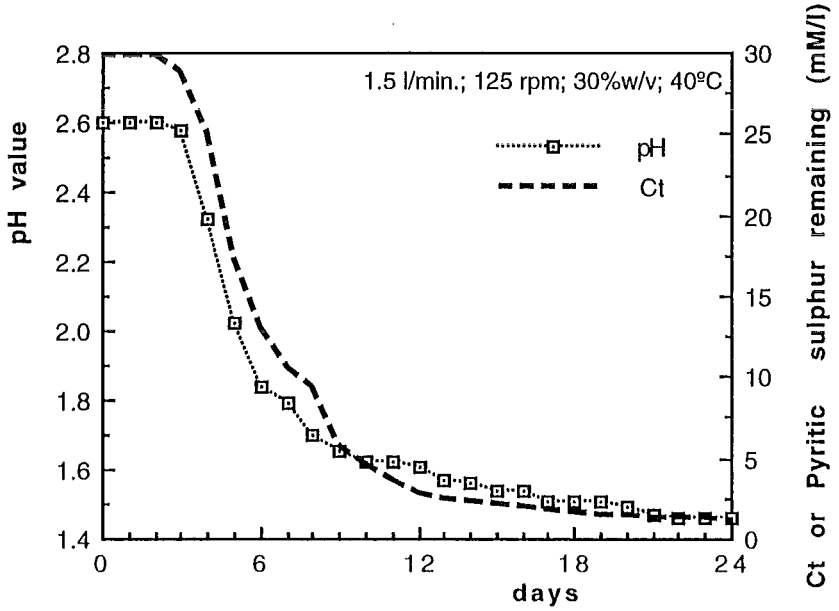


Figure 4. 2 : Profile pyritic sulphur remaining and pH value for an experiment conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration of 30%w/v and temperature of 40^{o}C.

Figure 4.2. shows a typical relationship of pyritic sulphur remaining and pH value in an experiment conducted using an air flow rate of 1. 5

litre/minute, stirrer speed of 125 rpm, slurry concentration of 30%w/v and temperature of 40°C.

pH equation

In order to correlate pH and pyritic sulphur content, pH values obtained from experiments and pyritic sulphur content (based on the coal samples analysis) have been collected and examined. A correlation between pH and pyritic sulphur concentration obtained by plotting pH_t/pH_o versus C_t/C_o has been made. Their correlation has been determined and an equation incorporates pH and pyritic sulphur concentration has been developed. The correlation is calculated in the case of one temperature and one slurry concentration.

Figure 4.3. represents an example plot of C_t/C_o versus pH_t/pH_o observed in an experiment using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration of 30%w/v and temperature of 40°C. The same procedure was also applied for the other experimental data.

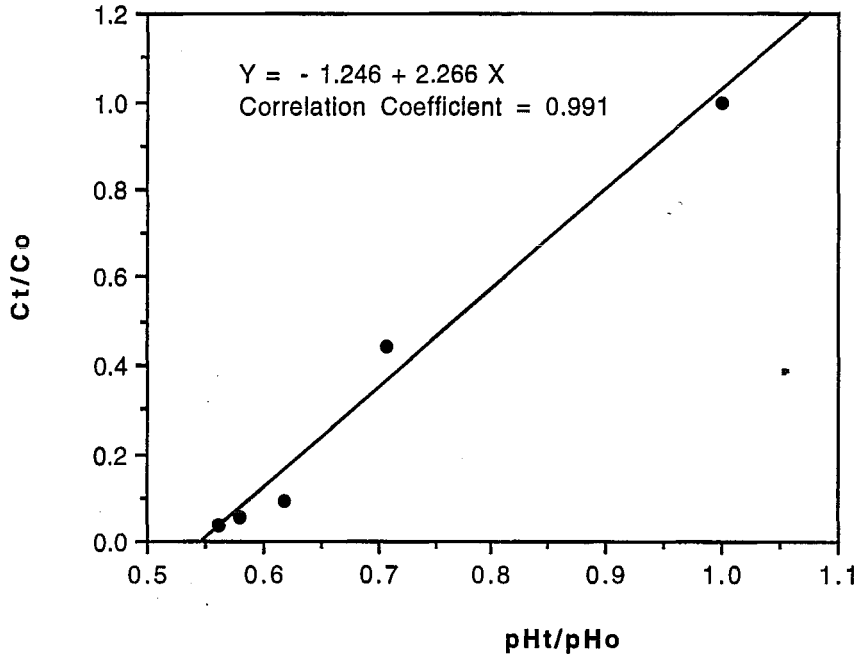


Figure 4.3 : Plot C_t/C_o versus pH_t/pH_o for an experiment conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration of 30%w/v and temperature of 40°C.

The general form of this equation is:

$$C_t / C_o = \partial + ß * (pH_t/pH_o)$$

This equation shows that the ratio of the concentration of pyritic sulphur remaining at time t to the original pyritic sulphur concentration is proportional to the ratio of pH at time t to the pH at time zero,
where:

C_t is the concentration of pyritic sulphur (mM/l) at time t (day);
C_o is the initial concentration of pyritic sulphur (mM/litre);
pH_t is the pH value of the medium at time t and
pH_o is the initial pH of the medium.

At $t = 0 \longrightarrow C_t / C_o = 1$ and $pH_t/pH_o = 1$,

i.e. $1 = \partial + ß * 1$

or $\partial + ß = 1$

From $Y = - 1.246 + 2.266 X$

$Y = 1$

Right hand side = 1.020, showing a good consistency. If desired, the equation could be modified to use this relationship between ∂ and ß.
Note also that when the concentration of pyrite approaches zero, $pH_t/pH_o = 0.55$.

This pH equation allows on line monitoring of the extent of coal desulphurisation as long as the pH of medium is constantly measured. This can be used to estimate the amount of pyritic sulphur removal during the process although it is realised that this pH equation is only a simplified estimation. However, this provides a very quick, practical indirect method for assessing the extent of sulphur removal.

The constants ∂ and ß for each pH equation obtained from data fitting are shown in tables 4.4 to 4.6.

Table 4.4.:

Constants ∂ and ß for pH equation in experiments with an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.

Temperature (° C)	coal slurry %w/v	Constants for pH equation ∂	ß	Correlation Coefficient
30	15	-1.0106	+1.8509	0.899
	20	-1.0053	+1.8870	0.946
	25	-0.9026	+1.7196	0.904
	30	-1.1618	+2.1383	0.989
	35	-0.4137	+1.4032	0.907
35	15	-1.9835	+2.9500	0.990
	20	-1.7882	+2.7497	0.991
	25	-1.6367	+2.6253	0.992
	30	-1.6061	+2.5823	0.995
	35	-1.3046	+2.2919	0.990
40	15	-1.1767	+1.9655	0.894
	20	-0.9181	+1.7267	0.907
	25	-1.0459	+2.0078	0.997
	30	-0.9167	+1.8034	0.973
	35	-0.8549	+1.7295	0.962
45	15	-1.4648	+2.4165	0.990
	20	-1.4855	+2.4140	0.928
	25	-1.1628	+2.1942	0.996
	30	-1.1459	+2.1695	0.997
	35	-0.8615	+1.8835	0.973

Table 4.5.:

Constants ∂ and ß for pH equation in experiments with an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

Temperature (°C)	coal slurry %w/v	Constants for pH equation ∂	ß	Correlation Coefficient
30	15	-2.0644	+2.9293	0.952
	20	-1.7204	+2.5842	0.950
	25	-1.8014	+2.7497	0.992
	30	-1.5795	+2.5633	0.996
	35	-1.6167	+2.5992	0.996
35	15	-1.9614	+2.9149	0.992
	20	-1.6832	+2.6984	0.998
	25	-1.4813	+2.4538	0.995
	30	-1.4503	+2.4539	0.999
	35	-1.3532	+2.2972	0.983
40	15	-1.8084	+2.7866	0.992
	20	-1.5284	+2.4875	0.988
	25	-1.3902	+2.3801	0.997
	30	-1.2463	+2.2656	0.991
	35	-1.1795	+2.2090	0.993
45	15	-1.4285	+2.4140	0.995
	20	-1.4054	+2.4306	0.988
	25	-1.1149	+2.1381	0.994
	30	-1.0213	+2.0436	0.994
	35	-1.0413	+2.0406	0.998

Table 4.6.:

Constants ∂ and ß for pH equation in experiments with an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

Temperature (° C)	coal slurry %w/v	Constants for pH equation ∂	ß	Correlation Coefficient
30	15	-2.3761	+3.2668	0.975
	20	-1.6213	+2.6097	0.918
	25	-1.7995	+2.7842	0.991
	30	-1.7319	+2.7903	0.993
	35	-1.5275	+2.5435	0.997
35	15	-1.9345	+2.8800	0.991
	20	-1.5713	+2.5601	0.998
	25	-1.4392	+2.4629	0.996
	30	-1.4584	+2.5250	0.988
	35	-1.1955	+2.1599	0.996
40	15	-1.5735	+2.5333	0.990
	20	-1.4162	+2.4062	0.999
	25	-1.3081	+2.3078	0.999
	30	-1.1170	+2.1646	0.978
	35	-1.0836	+2.1440	0.973
45	15	-1.4243	+2.4510	0.989
	20	-1.2444	+2.2354	0.998
	25	-1.0100	+2.0206	0.998
	30	-1.0647	+2.1127	0.983
	35	-1.0908	+2.1320	0.993

From these tables, the constants ∂ and ß for each set of experimental conditions are as follows:

1. For experiments with the following conditions:
air flow rate of 1.0 litre/minute; stirrer speed of 125 rpm;
temperature range (30-45)°C; slurry concentration: (15-35)%w/v:
- the constant ∂ is within the range (-0.90 to -1.98) and
- ß is within the range (+1.40 to +2.95) with
- the correlation coefficient (R) in the range (0.894 -0.997).

2. For experiments with the following conditions:
air flow rate of 1.5 litre/minute; stirrer speed of 125 rpm;
temperature range (30-45)°C; slurry concentration: (15-35)%w/v:
- the constant ∂ is within the range: (-1.02 to -2.06) and
- ß is within the range (+2.04 to +2.92) with R in the range (0.950-0.999).

3. For experiments with the following conditions:
air flow rate of 1.5 litre/minute; stirrer speed of 175 rpm;
temperature range (30-45)°C; slurry concentration: (15-35)%w/:
- the constant ∂ is within the range (-1.01 to -2.38) and
- ß is within the range (+2.02 to 2.88) with R in the range (0.918-0.999).

With these equations the pyritic sulphur removed from the coal at time t can be estimated based on the pH of the medium at time t .

Comparison between predicted and experimental data

The comparison of predicted and experimental concentration of pyritic sulphur removal (C_t) for different experimental conditions are shown in figures 4.4 to 4.6.
From these figures, the plot of predicted against actual concentration of pyritic sulphur removal shows that the points are clustered about the expected line of fit. However, some particular data points do not fit the line well.
The calculation of pyritic sulphur removal based on pH equation can be seen in appendix F and G.

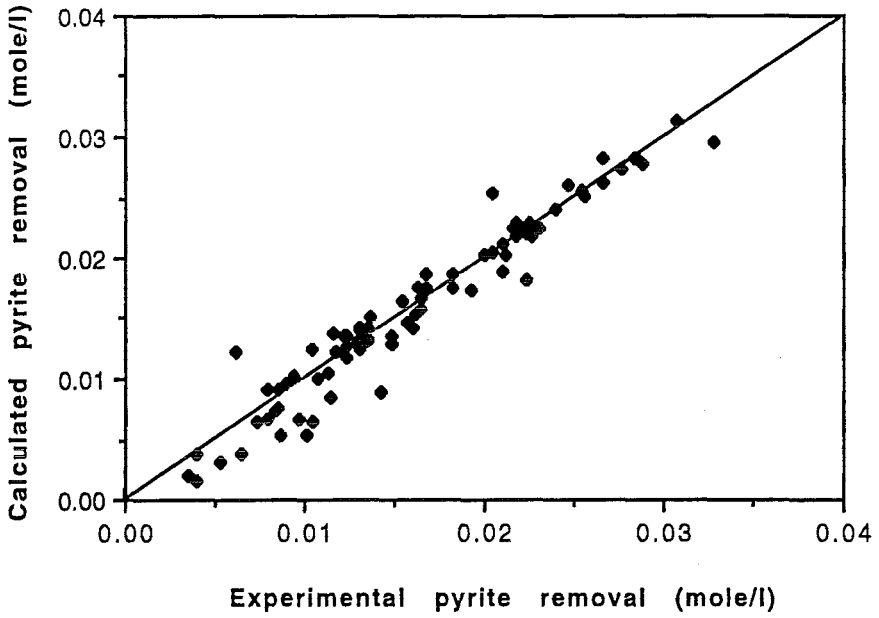


Figure 4. 4: Predicted vs experimental concentration of pyritic sulphur removal for experiments conducted using an air flow rate of 1.0 litre/minute, stirrer speed of 125 rpm, slurry concentration: (15-35)%w/v and temperature: (30-45)^{o} C (based on pH equation).

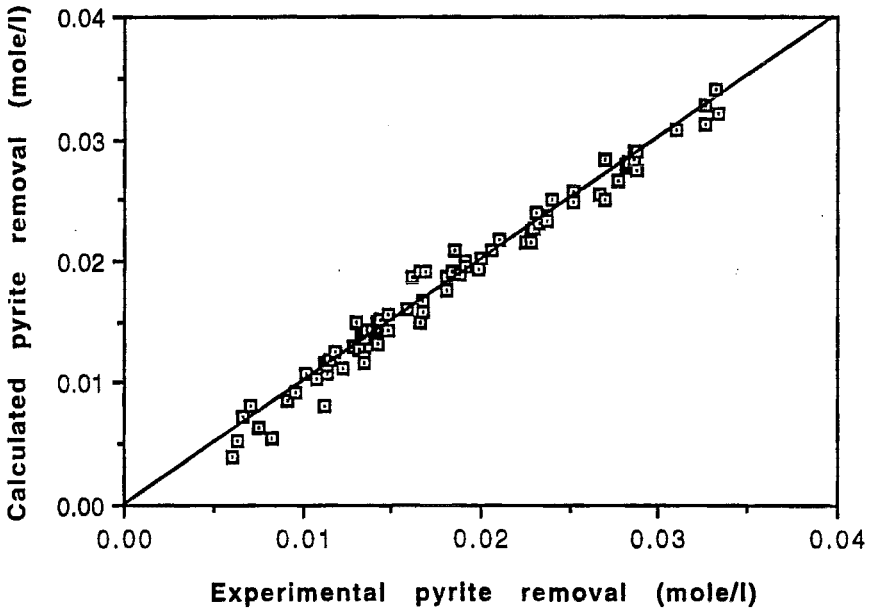


Figure 4. 5: Predicted vs experimental concentration of pyritic sulphur removal for experiments conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration: (15-35)% w/v and temperature: (30-45)^{o} C (based on pH equation).

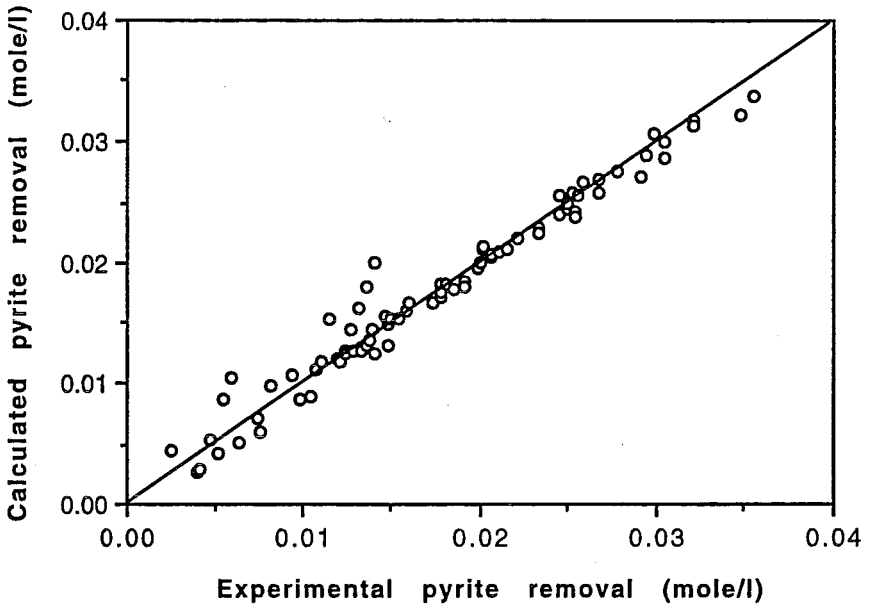


Figure 4. 6: Predicted vs experimental concentration of pyritic sulphur removal for experiments conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 175 rpm, slurry concentration: (15-35)% w/v and temperature: (30-45)^{o} C (based on pH equation).

The mean percentage discrepancies between the predicted and experimental data for:

1. Experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm are 6.2% with s = 5.7 (s = standard deviation).
2. Experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm are 3.8% with s = 3.3.
3. Experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm are 4.1% with s = 4.2.

Figure 4.7 shows an example of the comparison between the experimental and predicted pyritic sulphur removal calculated by using this pH equation.

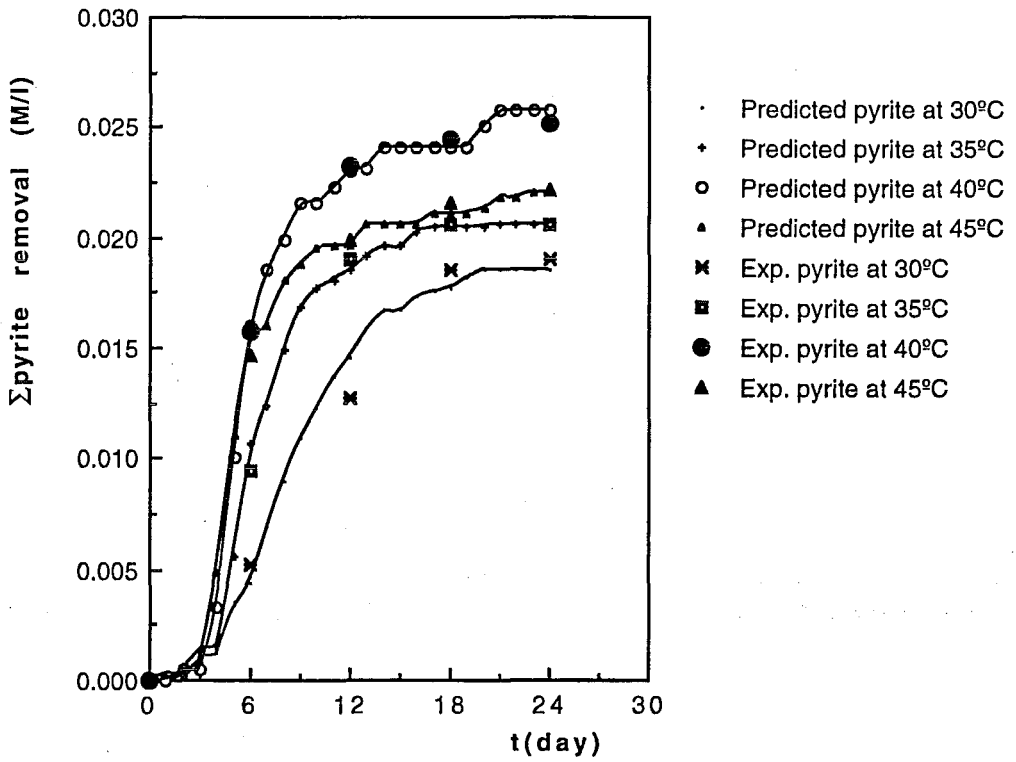


Figure 4. 7: Graph of predicted and experimental pyritic sulphur removal for experiments using an air flow rate of 1.5 litre/minute, stirrer speed of 175 rpm, slurry concentration of 25% w/v, temperature: (30-45)^{o}C.

This figure illustrates the result for the experiments at 30^{o}, 35^{o}, 40^{o} and 45^{o}C with air flow rate of 1.5 litre/minute, stirrer speed of 175 rpm and slurry concentration of 25% w/v. These show close coincidence between the experimental points with the predicted curve.
The predicted pyritic sulphur concentration used in this figure is presented in appendix H.

4.3. CONCLUSION

The observed pH values in this study indicated that pH of the medium changed from the initial pH of 2.6 to 1.4 during pyritic sulphur oxidation in Benneydale coal using *Thiobacillus* mixed culture. This result supports those of Dugan and Apel (1978) and Torma (1986) who mentioned that the optimal growth pH of *Thiobacilli* strain was within the range 1.5 to 3.5. More specifically, Rinder and Beier (1983); and Beyer *et al.* (1987a) said that the most suitable pH for sulphur removal from

coal using *Thiobacillus ferrooxidans* was about 1.8. The *Thiobacillus* mixed culture used in this study appeared to have a lower limit of pH 1.4. This pH was slightly lower than 1.5 which was considered (Torma, 1986) as the lowest optimal pH for good growth. This is not surprising because the optimum conditions for microbial growth also depend on the particular strain being used and the nature of the coal. Natural selection in adaptation stages and successive experiments appears useful for establishing a strain of microorganisms that is more tolerant to lower pH conditions.

The profile of pH values showed an exponential drop in the first 12 days of the process. The microorganisms were very active in that period and converted almost all of the pyritic sulphur present in the coal reaction medium. It also showed the lag phase within (1-2) days after the inoculation.

A metabolite of the microorganisms in this coal desulphurisation process is sulphuric acid. Attention should be given to subsequent treatment of this by-product. Spent reaction mixtures need to be treated before release into the environment. For example, the recovery of sulphuric acid from this acid water could be performed, or gypsum could be produced by addition of limestone (see appendix I). Effective engineering solutions to this problem is necessary to prevent environmental damage.

The pH and the pyritic sulphur concentration correlations developed in this study which has a form: $C_t / C_o = \partial + ß * (pH_t/pH_o)$ gave a reasonable estimation of the concentration of pyritic sulphur. With this method it is possible to obtain immediate information about the progress of pyritic sulphur oxidation at time t (shown by the amount of pyritic sulphur concentration) as long as the pH value is measured.

CHAPTER 5

KINETIC ASPECTS OF MICROBIAL COAL DESULPHURISATION

According to Torma and Panneton (1973), a kinetic analysis could elaborate the connection between metabolism and growth of the living organisms. This fundamental information would allow one to take maximum advantage of bacterial conversion, and also, suggest techniques which could lead to an increase in the yield of the desired product. However, the biokinetic modelling is especially difficult because of the many different metabolic pathways and side reactions involved which are important to the life of bacterial cell. Major complications in modelling come from the fact that many of the reaction mechanisms of the cell's metabolisms are not completely understood (Tsuchiya *et al.* 1966). The kinetic character of individual growth processes differs widely and a detail kinetic evaluation of experiments for microbial removal of pyrite in coal is lacking. Reaction rates for pyrite oxidation are reported only in a few cases. (Hoffman *et al.* 1981; Kargi and Robinson, 1982a, b; Beyer *et al.* 1986b).

5.1. ORDER OF REACTION

The order of reaction for pyritic sulphur removal at temperatures 30°, 35°, 40° and 45°C for different air flow rates and stirrer speeds has been determined from the log-log plot between the variables "pyritic sulphur removal rate" versus "substrate concentration". With respect to pyritic sulphur concentration this correlation indicated that the pyritic sulphur oxidation for Benneydale coal, was approximately a first order reaction .

5.1.1. VAN'T HOFF DIFFERENTIAL METHOD

The Van't Hoff differential method was used to calculate the order of reaction:

$$V = k \cdot C^n \qquad (5.1)$$

where: V is the rate of reaction (mole. L^{-1}. hour $^{-1}$); C is the substrate concentration (mole. L^{-1}); k is the reaction rate constant; and n is the order of reaction.

Linearising equation (5. 1) by taking the logarithm of both sides results in

$$\log V = \log k + n \log C \qquad (5.2)$$

which shows that the order of any particular reaction (n) is determined by the slope of a double logarithm linear plot of V against C.

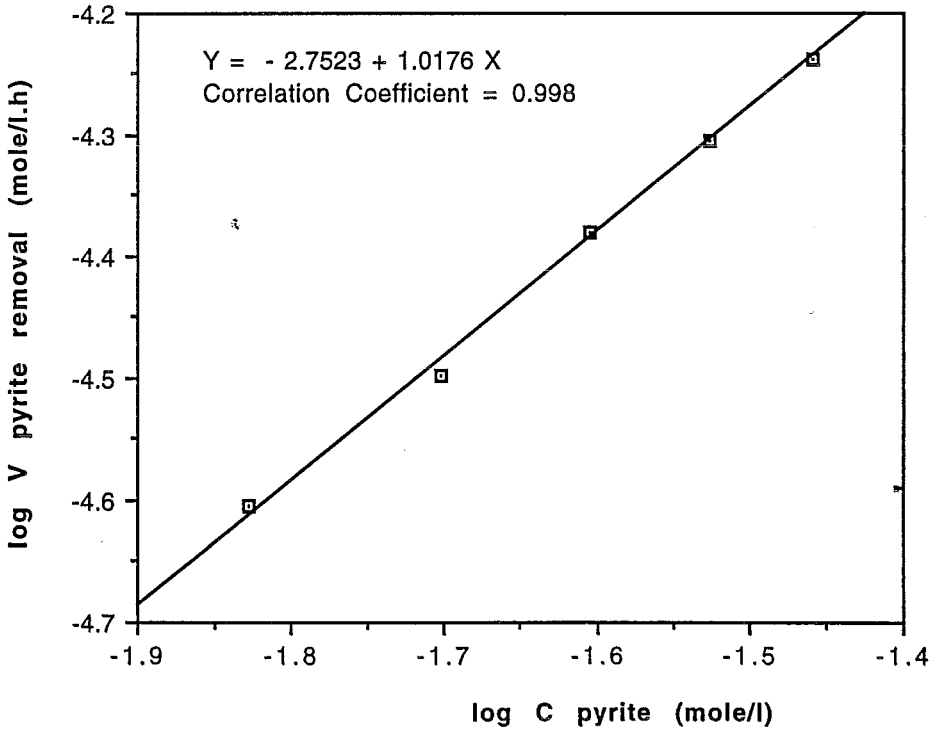


Figure 5. 1: Determination of order of reaction. Experimental conditions: air flow rate of 1.5 litre/minute; stirrer speed of 125 rpm and temperature of 40°C.

Figure 5.1 shows a typical plot of log (pyritic sulphur removal rate) versus log (concentration of pyritic sulphur) for experiments using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm and temperature of 40° C.
For the other experiments the plots are similar.

5.1.2. RESULTS AND DISCUSSION

The results are summarized in table 5.1 which shows the rate equations for the pyritic sulphur removal for Benneydale coal with respect to pyritic sulphur concentration under a variety of experimental conditions.

Table 5.1

Rate equations for pyritic sulphur removal with respect to pyritic sulphur concentration.

Experiment conditions	Rate of equation	Correlation Coefficient
30°C;1.0 l/min.;125 rpm	$V = 2.26 \times 10^{-3} \cdot C^{1.14}$	0.979
35°C;1.0 l/min.;125 rpm	$V = 1.13 \times 10^{-3} \cdot C^{0.91}$	0.984
40°C;1.0 l/min.;125 rpm	$V = 1.87 \times 10^{-3} \cdot C^{1.03}$	0.997
45°C;1.0 l/min.;125 rpm	$V = 2.47 \times 10^{-3} \cdot C^{1.11}$	0.988
30°C;1.5 l/min.;125 rpm	$V = 1.92 \times 10^{-3} \cdot C^{1.05}$	0.999
35°C;1.5 l/min.;125 rpm	$V = 1.40 \times 10^{-3} \cdot C^{0.96}$	1.000
40°C;1.5 l/min.;125 rpm	$V = 1.77 \times 10^{-3} \cdot C^{1.02}$	0.998
45°C;1.5 l/min.;175 rpm	$V = 1.95 \times 10^{-3} \cdot C^{1.05}$	0.987
30°C;1.5 l/min.;175 rpm	$V = 1.59 \times 10^{-3} \cdot C^{0.99}$	0.999
35°C;1.5 l/min.;175 rpm	$V = 1.42 \times 10^{-3} \cdot C^{0.96}$	0.999
40°C;1.5 l/min.;175 rpm	$V = 1.83 \times 10^{-3} \cdot C^{1.03}$	1.000
45°C;1.5 l/min.;175 rpm	$V = 1.61 \times 10^{-3} \cdot C^{0.99}$	0.999

The order of reaction (n) was found to be in the range (0.91-1.14); (0.96-1.05) and (0.96-1.03) for the experimental conditions given in table 5.1, with a correlation coefficient (R) in the range of (0.98-1.00).
These indicated that the rate of pyritic sulphur oxidation in this study was following, approximately, a first order with respect to pyritic sulphur concentration. These first order reaction rates agree with those obtained by earlier workers (Detz and Barvinchak (1979); Bos *et al.* (1986) and Kargi and Robinson (1985)).
On the other hand, Olsen *et al.* (1980) working with pure pyritic sulphur and coals from Ohio and New Mexico, USA, found the following rate equations:

for pure pyritic sulphur oxidation: $V = 0.05.\ C^{0.63}$

for oxidation of Ohio coal-pyritic sulphur: $V = 0.12.\ C^{0.53}$

for oxidation of New Mexico coal-pyritic sulphur: $V = 0.04.\ C^{0.73}$

These rate equations indicated that oxidation was not first order with respect to C as had earlier been reported. This disagreement is not surprising since it can be argued that the rate of pyritic sulphur dissolution should depend on differences in the pyritic sulphur content and degree of dissimination as well as associated mineralogical inclusions. Also, the desulphurisation rate could be expected to vary with coal rank and the degree of weathering of a particular coal.

5.2. KINETIC MODEL OF PYRITIC SULPHUR REMOVAL FROM COAL

An approximately first order rate of reaction with respect to pyritic sulphur concentration has been found and based on this assumption the rate constants (k_c) for the kinetic equations have been determined.

According to Voznaya (1981) activation energy is the minimum excess energy compared with the mean energy of the reacting molecules at a given temperature, which the molecules need to possess if their collision is to produce a new product. The dependence of reaction rate constant on temperature is merely an extension of the kinetic theory of matter. As thermal energy increases (thereby increasing electron spin) the concentration of molecules with a higher level of energy rises.

A more accurate dependence of the velocity constant on temperature is described by the Arrhenius equation obtained by formal analysis of the Van't Hoff isochore equation. The Arrhenius parameters include the activation energy E_a and the pre-exponential or frequency factor A which characterize the Arrhenius equation. The activation energy E_a is related to the reaction velocity or rate constant k_c by the empirical expression:

$$k_c = A \exp. (- E_a / RT)$$

This equation is first used by Arrhenius, where:

R and T are the universal gas constant and absolute temperature, respectively.

A is the pre-exponential (or the frequency) factor and has the same units as k_c.

E_a was considered by Arrhenius to be the amount of energy in excess of the average energy level which the reactants must have in order for the reaction to proceed. The equation shows that the constant characterizing the reaction are the pre-exponential factor A and the activation energy E_a. The higher is E_a, the lower (at a given A) the rate of a chemical reaction. The activation energy (E_a) can be determined from the plot of variable log k_c versus 1/T.

The kinetics of pyritic sulphur conversion in this study can be described by the following rate equation:

$$-dC_{pyrite} / dt = A \exp. (- E_a / RT) * C_{pyrite}$$

which shows that the rate of reaction of pyrite is proportional to the concentration of pyrite times the "exponential activation energy/temperature" expression. This kinetic equation can be used to predict the rate of pyritic sulphur conversion throughout the desulphurisation process.

5.2.1. LITERATURE REVIEW

In this part, a review of several kinetic models of the pyritic sulphur oxidation is presented. Various authors have used different approaches to describe their kinetic models of pyritic sulphur oxidation.

In 1979, Detz and Barvinchak developed a first order rate equation which assumed that the pyritic sulphur was completely accessible to microorganisms. They reported rate constants in the range of (2.0×10^{-3} - 2.5×10^{-2}) h^{-1} for *Thiobacillus ferrooxidans* . However, Olsen *et al.* (1980) determined the order of the reaction to be less than 1, namely 0.63; 0.53 and 0.73 for pure pyrite, for Ohio and New Mexico coal respectively.

Huber *et al.* (1983) suggested a first order reaction constant for the substrate limited regime model. The model described leaching experiments with two coal samples, which were monitored for both oxygen and carbon dioxide uptake rates. These results were also confirmed by monitoring oxygen consumption rates (Beyer *et al*, 1986 and Hone *et al.* 1987).

Hoffman *et al.* (1981); Hone (1988) and Uhl *et al.* (1988) mentioned that the rate of reaction at the surfaces of solid particles was first order for accessible pyritic sulphur surface areas. In this case due to the heterogeneous physical structure of the coal, the determination of the true surface area may cause problems. Rate constants in the range of (0.018-1.25) mg FeS_2 per cm^2 pyritic sulphur surface per day have been found.

A bacterial growth model based on adsorption/desorption kinetics which considered bacterial growth in solution as well as on the pyritic sulphur surface, has been developed by Chang and Myerson in 1982. In their model they used pure iron pyritic sulphur with *Thiobacillus ferrooxidans*. Applying this model to a coal desulphurisation process, Myerson and Kline (1983, 1984) claimed consistent agreement with their experimental results.

An extended adsorption/desorption model which considered selective attachment of bacteria to pyritic sulphur surfaces was introduced by Kargi and Wiessman in 1984. Their calculations were in good agreement with literature data on the effect of initial substrate concentration, coal slurry concentration, particle size and initial cell concentration.

Andrews and Maczuga (1984) established a model which considered the physical structure of the coal. In order to describe the kinetics of the

microbial desulphurisation process, this model was based on flat plate geometry of the coal particles and a distribution function for the size of pyritic sulphur inclusions. The model had only two unknown parameters, i.e. the velocity constant (μm day^{-1}) and the inclusion length (μm) which could easily be derived from a simple batch experiment. Applying this model, Hone (1988) working with several coal types could only find agreement in one case. For other coal qualities his approach failed due to effects of small particle size and the presence of considerable amounts of pyritic sulphur liberated from the coal.

5.2.2. KINETIC RATE CONSTANT (k_c)

The relation between the rate of pyritic sulphur converted and the pyritic sulphur concentration in this study has been found to correlate approximately with a first order rate equation, which implies that the rate expression for the equation is:

$$- V_A = - dC_A / dt = k_c C_A \qquad (5.3)$$

Integration of equation (5. 3) gives:

$$\ln C_{Ao} / C_{At} = k_c t \qquad (5.4)$$

where:

C_{Ao} and C_{At} are the initial and instantaneous concentrations of pyritic sulphur (mole/litre), respectively;
k_c is the rate constant and
t is the time.

Validity of equation (5.4) is shown in figures 5.2.
The slope of this plot being the value of k_c a fairly straight line shows close agreement with the assumed model equation.

For the other experiments the same plotting procedure was applied (results can be seen in appendix J).

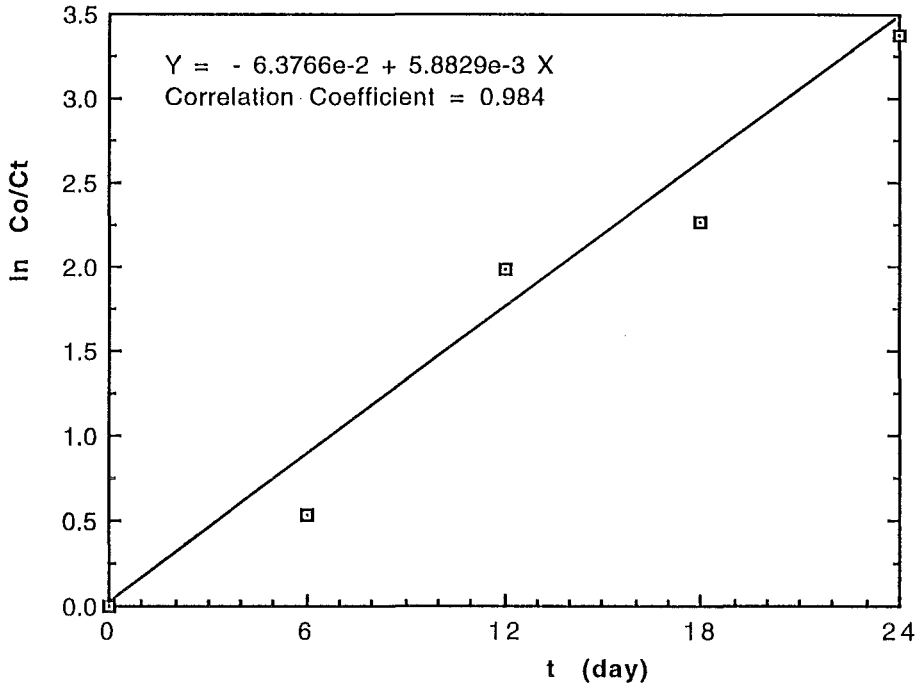


Figure 5. 2: Plot of ln C_o/C_t versus time, for an experiment conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 175 rpm, slurry concentration of 20% w/v and temperature of 35°C.

The rate constant k_c values are shown in tables 5.2 - 5.4.
From these tables it was found that the rate kinetic constants (k_c) were as follows:
(1). within the range of (1.9×10^{-3} - 1.3×10^{-2}) h^{-1} with correlation coefficient in the range of (0.899-0.995) for the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.
(2). within the range of (3.2×10^{-3} - 6.9×10^{-3}) h^{-1} with correlation coefficient in the range of (0.907-0.995) for the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.
(3). within the range of (5.0×10^{-3} - 7.4×10^{-3}) h^{-1} with correlation coefficient in the range of (0.928-0.996) for the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

Table 5.2

Rate constant (k_c) for coal desulphurisation experiments conducted using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.

Slurry conc. (w/v)	Temp. (° C)	Rate constant (k_c) ($hour^{-1}$)	Correlation Coefficient
15 %	30	1.9048 e-3	0.923
	35	5.8633 e-3	0.964
	40	4.5823 e-3	0.982
	45	5.1624 e-3	0.938
20%	30	2.4825 e-3	0.978
	35	5.2371 e-3	0.982
	40	3.9826 e-3	0.980
	45	2.6831 e-3	0.954
25%	30	3.1366 e-3	0.970
	35	1.3367 e-2	0.899
	40	6.5177 e-3	0.982
	45	5.8294 e-3	0.988
30%	30	3.5107 e-3	0.964
	35	5.1991 e-3	0.981
	40	5.7656 e-3	0.995
	45	7.5470 e-3	0.986
35%	30	2.1680 e-3	0.946
	35	3.0094 e-3	0.986
	40	5.2478 e-3	0.990
	45	6.1935 e-3	0.954

Table 5.3

Rate constant (k_c) for coal desulphurisation experiments conducted using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

Slurry conc. (w/v)	Temp. (° C)	Rate constant (k_c) ($hour^{-1}$)	Correlation Coefficient
15%	30	4.0540 e-3	0.987
	35	6.7826 e-3	0.981
	40	5.6269 e-3	0.907
	45	6.0607 e-2	0.964
20%	30	3.9057 e-3	0.975
	35	5.6377 e-3	0.949
	40	4.4062 e-3	0.912
	45	3.2228 e-3	0.986
25%	30	5.0087 e-3	0.972
	35	5.6193 e-2	0.969
	40	6.3461 e-3	0.980
	45	5.6258 e-3	0.945
30%	30	5.2615 e-3	0.978
	35	6.1132 e-3	0.966
	40	5.9657 e-3	0.970
	45	6.8547 e-3	0.960
35%	30	5.2680 e-3	0.973
	35	5.4940 e-3	0.950
	40	5.6215 e-3	0.995
	45	6.3169 e-3	0.930

Table 5.4

Rate constant (k_c) for coal desulphurisation experiments conducted using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

Slurry conc. (w/v)	Temp. (^{o} C)	Rate constant (k_c) ($hour^{-1}$)	Correlation Coefficient
15%	30	5.0503 e-3	0.978
	35	5.6900 e-3	0.976
	40	5.0813 e-3	0.965
	45	6.9165 e-3	0.959
20%	30	5.0255 e-3	0.944
	35	5.8829 e-3	0.984
	40	5.7932 e-3	0.963
	45	5.7065 e-3	0.928
25%	30	6.1737 e-3	0.971
	35	4.9481 e-2	0.955
	40	5.5326 e-3	0.982
	45	5.0261 e-3	0.985
30%	30	5.1492 e-3	0.978
	35	5.4101 e-3	0.937
	40	5.9911 e-3	0.996
	45	5.9106 e-3	0.948
35%	30	5.1064 e-3	0.967
	35	5.2264 e-3	0.985
	40	6.2440 e-3	0.988
	45	7.3739 e-3	0.954

These k_c values were found to be consistent with the results of other workers, such as Detz and Barvinchak (1979); Andrews and Maczuga (1982); Huber *et al.* (1984) and Hone (1988) who were dealing with a first order reaction in pyritic sulphur concentration in their kinetic models.

Detz and Barvinchak (1979) found the k_c value was within the range of (6.0×10^{-3} - 14.4×10^{-3}) h^{-1} in their experiment using *Thiobacillus ferrooxidans* , at 28°C and a coal of particle size 74 μm.
In 1982, Andrews and Maczuga, calculated the k_c value was within the range of (2.0×10^{-3} - 6.0×10^{-3}) h^{-1} for coal desulphurisation at 25°C using coal of particle diameter (2-50)μm.
At 30°C, Huber *et al.* (1984) estimated a rate constant of 2.5×10^{-3} (h^{-1}).
In his study, Hone (1988) stated that the k_c value was 3.3×10^{-3} (h^{-1}) for coal desulphurisation at 25°C using coal of particle diameter 25 μm (see table 5.5.)

Table 5.5

Value of k_c in other kinetic models

Kinetic model	Parameter k_c (h^{-1}) to be estimated	Experimental conditions	References
1st order reaction in pyritic sulphur conc. $V=k_c.S_c$	6.0×10^{-3} - 14.4×10^{-3}	*Thiobacillus ferrooxidans* Dp= 74 µm T= 28° C	Detz and Barvinchak (1979)
	2.0×10^{-3} - 6.0×10^{-3}	*Thiobacillus ferrooxidans* Dp= (2-5)µm T= 25° C	Andrews and Maczuga (1984)
	2.0×10^{-3}	*Sulfolobus acidocaldarius* Dp= 48 µm T= 70° C	Kargi and Robinson (1985)
	2.5×10^{-3}	*Thiobacillus ferrooxidans* Dp=powder* T= 30° C	Huber *et al.* (1984)
	3.3×10^{-3}	*Thiobacillus ferrooxidans* Dp= 25 µm T= 25° C	Hone (1979)

Dp= particle size of coal sample, (*) not stated

5.2.3. ACTIVATION ENERGY

Activation energy E_a can be determined experimentally by measuring the reaction velocity as a function of temperature (Montgomery and Swenson, 1976). If ln k_c is plotted versus 1/T, the slope line is E_a/R (or log k_c is plotted versus 1/T, the slope of the line is $-E_a/2.3\ R$).

In this study the energy activation (E_a) is determined from the plot of variable log k_c versus (1/T). An example of this plot is presented in figure 5.3, which shows a plot of the log k_c versus (1/T) for an experiment with an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm and slurry concentration of 35% w/v.

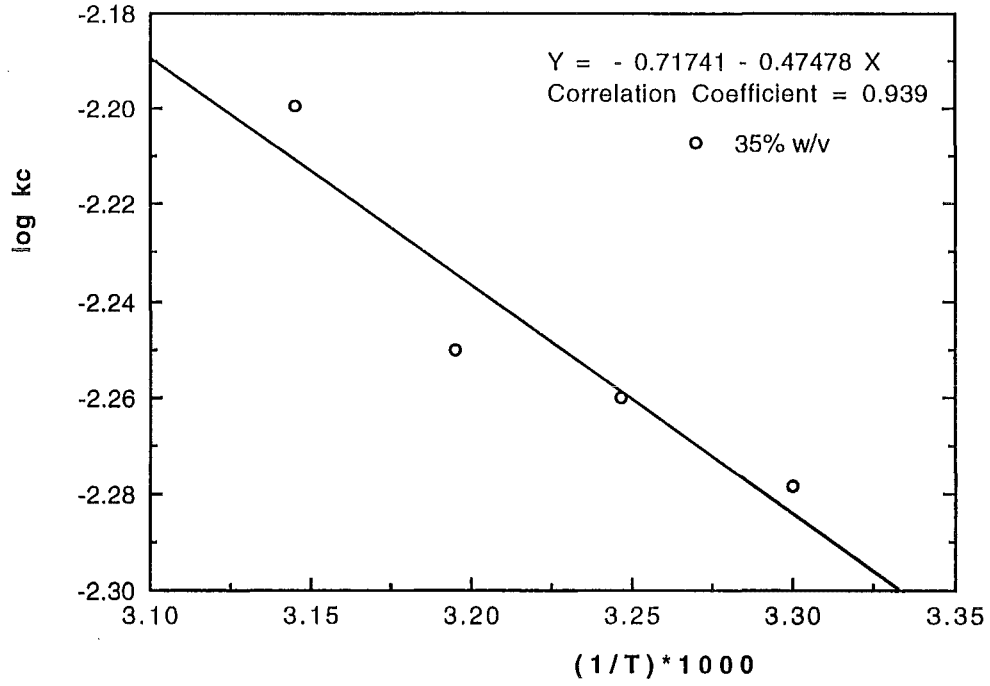


Figure 5. 3: Plot log k_c versus 1/T, for an experiment conducted using an air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration of 35% w/v, temperature range: (30-45)°C.

The same procedure was applied for a variety of experimental conditions using different temperatures, air flow rates, stirrer speeds and slurry concentrations. The results can be seen in appendix K.

The activation energy E_a was calculated based from those figures (see appendix L) and these are summarized in table 5.6.

Table 5.6

The activation energy E_a for pyritic sulphur oxidation in Benneydale coal

Experimental-conditions	E_a (kcal/mole)	Correlation Coefficient
15%w/v, 1 l/min., 125 rpm	10.6	0.701
20%w/v, 1 l/min., 125 rpm	8.9	1.000
25%w/v, 1 l/min., 125 rpm	8.8	0.895
30%w/v, 1 l/min., 125 rpm	9.2	0.977
35%w/v, 1 l/min., 125 rpm	14.2	0.983
15%w/v, 1.5 l/min., 125 rpm	5.3	0.989
20%w/v, 1.5 l/min., 125 rpm	2.3	1.000
25%w/v, 1.5 l/min., 125 rpm	4.5	1.000
30%w/v, 1.5 l/min., 125 rpm	2.9	0.915
35%w/v, 1.5 l/min., 125 rpm	2.2	0.939
15%w/v, 1.5 l/min., 175 rpm	4.0	0.999
20%w/v, 1.5 l/min., 175 rpm	2.7	0.822
25%w/v, 1.5 l/min., 175 rpm	4.3	1.000
30%w/v, 1.5 l/min., 175 rpm	2.9	0.979
35%w/v, 1.5 l/min., 175 rpm	4.9	0.960

As the activation energy is a function of the molecular state and the temperature, it seems unlikely that increased mixing (oxygen access) can affect it. What is tabulated above is probably better described as an "apparent activation energy".

Table 5.6 shows that E_a values obtained in experiments conducted using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm (range from 8.8 to 14.2 kcal/mole) were higher than those obtained in experiments conducted using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm (range from 2.2 to 5.3 kcal/mole). Higher E_a meant that the rate of reaction was slower. So, in this case, increasing air flow rate from 1.0 litre/minute to 1.5 litre/minute improved the rate of pyritic sulphur removal which supports the earlier finding (page 33 and 52). On the other hand, increasing stirrer speed from 125 rpm to 175 rpm (with air flow rate of 1.5 litre/minute) only showed a negligible change in E_a values which indicated that there was no clear advantage of increasing stirrer speed from 125 rpm to 175 rpm as shown previously (page 36 and 52).

5.2.4 KINETIC RATE EQUATION FOR PYRITIC SULPHUR CONVERSION

A general rate expression for pyritic sulphur conversion in this study has a form:

$$V_{pyrite} = f\ (C_{pyrite};\ T;\ t) \qquad (5.6)$$

where:

V_{pyrite} is the rate of pyrite conversion (mole/l. h),
C_{pyrite} is the concentration of pyrite at time t (mole/l),
T is the absolute temperature and
t is the time (hour).

The first order rate expression for the kinetic equation is:

$$V_{pyrite} = -\ dC_{pyrite} / dt = k_c\ C_{pyrite} \qquad (5.7)$$

$$k_c = A \exp.\ (-\ E_a / RT) \qquad (5.8)$$

so, the equation becomes:

$$-\ dC_{pyrite} / dt = A \exp.\ (-\ E_a / RT)\ (C_{pyrite}) \qquad (5.9)$$

where:

A is the pre-exponential rate,
E_a is the activation energy of pyrite reaction (cal/mole),
R is the gas constant (1.986 cal/(mole.^oK),
T is the temperature (^oK),
C_{pyrite} is the concentration of pyrite (mole/litre) and
t is the time

Tables 5.7 to 5. 9 show the rate equations for experiments using various air flow rates, stirrer speeds and slurry concentrations (E_a in cal/mole).

Table 5.7

Rate equations of pyritic sulphur conversion for experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm

slurry concentration %w/v	Rate equation: $-dC_{pyrite}/dt = k_c . C_{pyrite}$ or $-dC_{pyrite}/dt = A \exp.(-E_a/RT). C_{pyrite}$
15	$1.3 \times 10^5 \exp.(-10630.7/RT). C_{pyrite}$
20	$7.0 \times 10^3 \exp.(-8930.0/RT). C_{pyrite}$
25	$8.2 \times 10^3 \exp.(-8846.0/RT). C_{pyrite}$
30	$1.7 \times 10^4 \exp.(-9215.5/RT). C_{pyrite}$
35	$4.2 \times 10^7 \exp.(-14237.4/RT). C_{pyrite}$

Table 5.7 shows the rate equations for the experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.

Table 5.8

Rate equations of pyritic sulphur conversion for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm

slurry concentration %w/v	Rate equation: $-dC_{pyrite}/dt = k_c . C_{pyrite}$ or $-dC_{pyrite}/dt = A \exp.(-E_a/RT). C_{pyrite}$
15	$28.0 \exp.(-5307.8/RT). C_{pyrite}$
20	$0.2 \exp.(-2277.9/RT). C_{pyrite}$
25	$8.5 \exp.(-4470.1/RT). C_{pyrite}$
30	$0.7 \exp.(-2949.4/RT). C_{pyrite}$
35	$0.2 \exp.(-2168.7/RT). C_{pyrite}$

Table 5.8 shows the rate equations for the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

Table 5.9

Rates equation of pyritic sulphur conversion for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm

slurry concentration %w/v	Rate equation: $-dC_{pyrite}/dt = k_c . C_{pyrite}$ or $-dC_{pyrite}/dt = A \exp.(-E_a/RT). C_{pyrite}$
15	$3.9 \exp.(-3991.3/RT). C_{pyrite}$
20	$0.5 \exp.(-2696.1/RT). C_{pyrite}$
25	$5.3 \exp.(-4250.6/RT). C_{pyrite}$
30	$0.6 \exp.(-2857.8/RT). C_{pyrite}$
35	$2.7 \exp.(-4896.7/RT). C_{pyrite}$

Table 5.9 shows the rate equations for the experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm.

5.2.5. COMPARISON BETWEEN PREDICTED AND EXPERIMENTAL DATA

In this section estimation of the amount of pyritic sulphur removal based on the kinetic equations above is presented.

Figures 5.4. - 5.6. show the comparison between predicted and experimental concentration of pyritic sulphur removal obtained in the experiments conducted using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm; 1.5 litre/minute and 125 rpm and 1.5 litre/minute and 175 rpm, respectively.

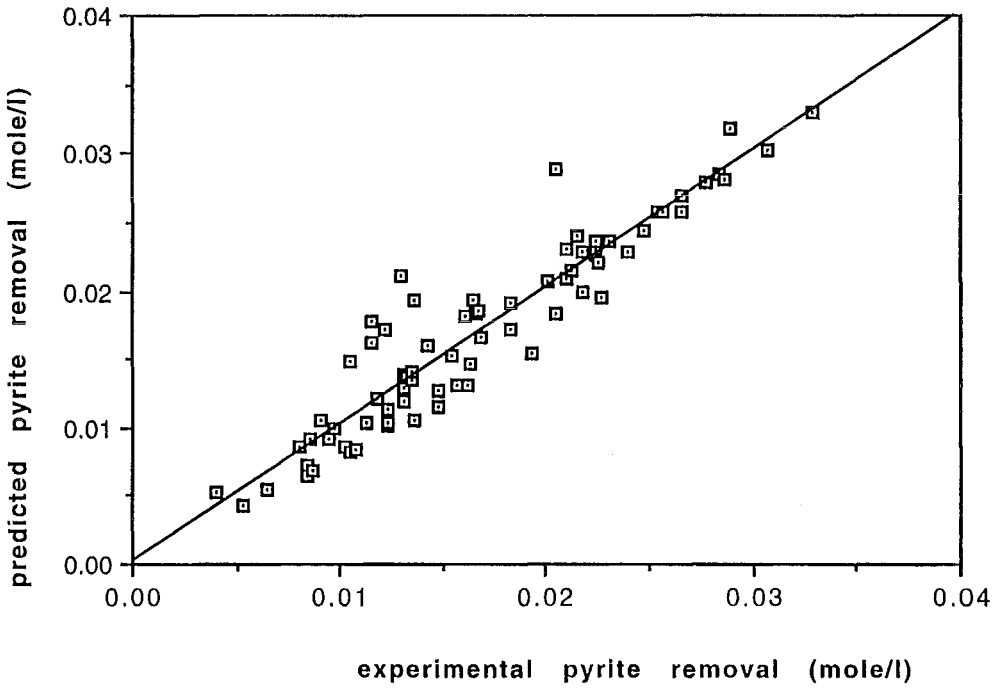


Figure 5. 4: Predicted vs experimental concentration of pyritic sulphur removal for experiments using an air flow of 1.0 litre/minute, stirrer speed of 125 rpm, slurry concentration (15-35)% w/v, and temperature: (30-45)^{o} C (based on kinetic equation).

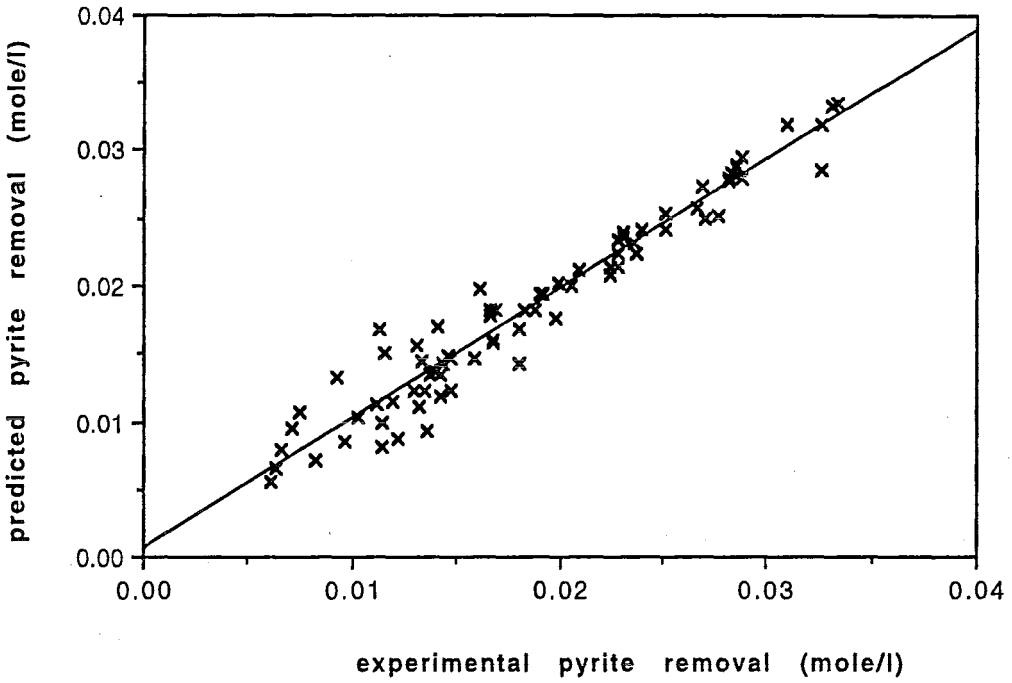


Figure 5. 5: Predicted vs experimental concentration of pyritic sulphur removal experiments using an air flow of 1.5 litre/minute, stirrer speed of 125 rpm, slurry concentration (15-35)% w/v, and temperature: (30-45)^{o} C (based on kinetic equation).

These figures show points closely clustered around an ideal line which shows that the predicted data fit quite well with the exception of a few data points

The mean difference between the predicted and experimental concentration of pyritic sulphur removal were: 14.5%; 9.4% and 16.5% with standard deviation: 14.8; 10.7; and 48.0, respectively, for these experiments.

These results indicated that the proposed kinetic equation was quite accurate for the estimation of pyritic sulphur removal in this study.

In appendix M the predicted and the experimental concentration of pyritic sulphur removal are presented.

The calculation of predicted pyritic sulphur removal based on the equations can be seen in appendix O.

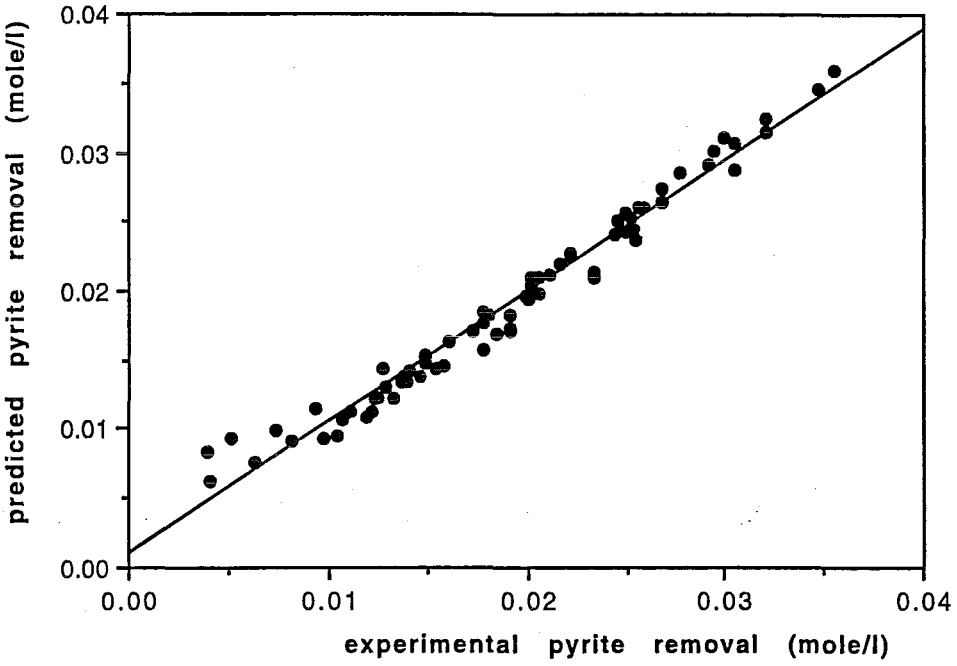


Figure 5. 6: Predicted vs experimental concentration of pyritic sulphur removal experiments using an air flow of 1.5 litre/minute, stirrer speed of 175 rpm, slurry concentration (15-35)% w/v and temperature: (30-45)^{o}C (based on kinetic equation).

Figure 5.7. compares the predicted and experimental results for the amount of pyritic sulphur removal for experiments using an air flow rate of 1.5 litre/minute, stirrer speed of 175 rpm, within a temperature range (30-45)^{o}C and slurry concentration of 25% w/v. Some experimental points fit well with the line of the predicted pyritic sulphur removal derived from the kinetic equation, although some do not. The degree of fit varies from a close fit for curve at 40^{o}C to wide scatter for curve at 30^{o}C. In this study samples of coal were taken at 6 day intervals. From the experimental data it was recognized that a smaller sampling interval during the first 12 days of the process would have helped, because most of the pyritic sulphur removal had occurred in this period. The rate of pyritic sulphur removal followed a steep exponential curve initially for the first 12 days up to a reasonably level plateau thereafter.
A closer sampling would have led to a more accurate testing of the kinetic model.

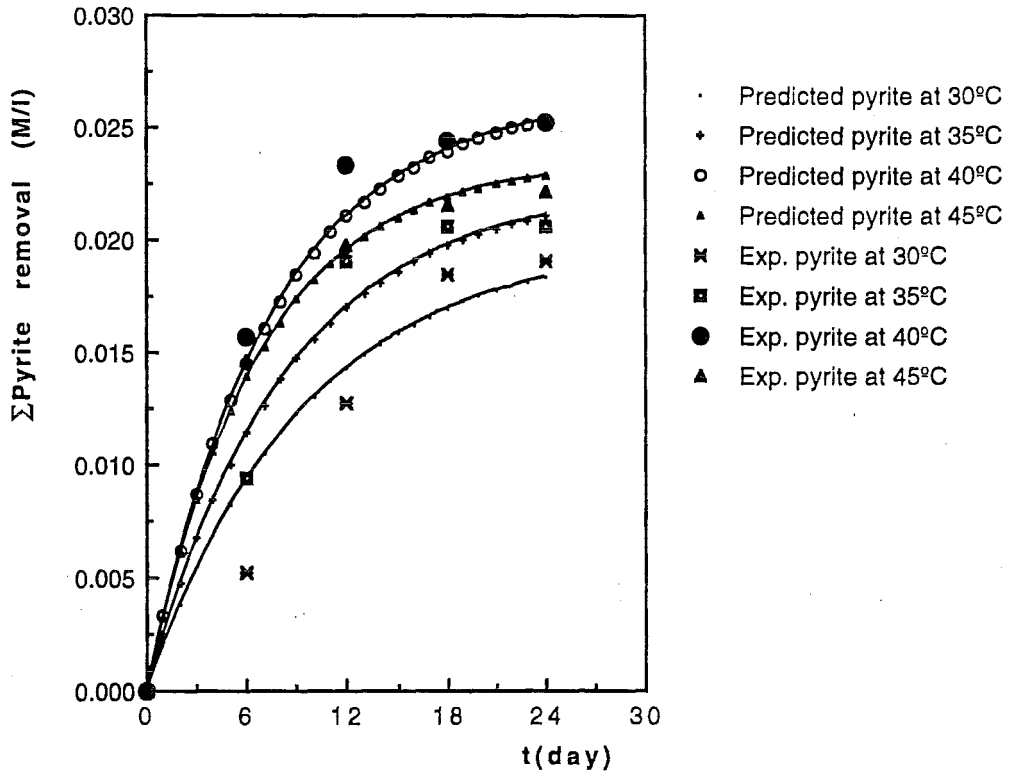


Figure 5. 7: Typical graphs predicted and experimental pyritic sulphur removal for experiment using an air flow rate of 1.5 litre/minute, stirrer speed of 175 rpm, slurry concentration of 25% w/v, and temperature: 30°; 35°; 40° and 45° C

5.3. CONCLUSION

The rate of pyritic sulphur removal in this study followed, approximately, a first order with respect to pyritic sulphur concentration. The kinetic rate constant (k_c) in this study has been determined and values were as follows: within the range of ($1.9 \times 10^{-3} - 1.3 \times 10^{-2}$) h^{-1} for experiments using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm; within the range of ($3.2 \times 10^{-3} - 6.9 \times 10^{-3}$) h^{-1} for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm; and within the range of ($5.0 \times 10^{-3} - 7.4 \times 10^{-3}$) h^{-1} for experiments using an air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm. These k_c values were found to be consistent with the results of other workers,

such as Detz and Barvinchak (1979); Andrews and Maczuga (1982); Huber *et al.* (1984) and Hone (1988).

The activation energy (E_a) has also been determined by plotting log k_c versus temperature. The activation energy for this microbial pyritic sulphur removal is within the range (8.8 - 14.2) kcal/mole; (2.2 - 5.3) kcal/mole; and (2.7 - 4.9) kcal/mole, respectively, for the experimental conditions in table 5.6. These show that the reaction rate in the experiments conducted using an air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm is the slowest one.

The predicted concentration of pyritic sulphur removal correlated quite well with the experimental data. The kinetic equation could have been tuned better if the sampling interval for sulphur determination had occurred more frequently. A better sampling frequency would have been on a daily basis especially during the first 12 days of the experiment. After this, sampling could have occurred every 6 days.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

The performance of the *Thiobacillus* mixed culture isolated from acid mine water was studied in the laboratory using stirred batch reactors. Results showed that the mixed culture had the ability to convert (92%-95%) of the pyritic sulphur in Benneydale coal within 12 days.
The performance of this culture was affected by several variables, i.e. coal slurry concentration, air flow rate, stirrer speed and temperature. For desulphurisation at high slurry concentrations (i.e. 35% w/v) increasing air flow rate from 1.0 litre/minute to 1.5 litre/minute (at 125 rpm of stirrer speed) had a significant effect on the rate of pyritic sulphur conversion. On the other hand, increase of stirrer speed from 125 rpm to 175 rpm had little or no effect on the performance of this culture. The stirrer speed of 125 rpm was sufficient for maintaining the efficiency of the process. The results of these experiments suggest that gas mass transfer, mixing and aeration are essential requirements for a bioprocess which needs close investigation especially when high volumetric productivity is important.

It was found that the optimum temperature for this *Thiobacillus* mixed culture ranged from 35°C to 40°C. It is recommended that the temperature of the bioprocess using this culture is set in this range to ensure an optimum result.

The pH method $C_t/C_o = \partial + ß * (pH_t/pH_o)$ to estimate pyritic sulphur concentration during the experiments was found to give a good result. It

was simple and rapid. In this study pH has proved to be a suitable indicator for indirectly determining the rate of pyritic sulphur removal, although further work using coal with higher sulphur content is required.

Using an approximately first order rate of reaction with respect to initial pyritic sulphur concentration, a kinetic rate of pyritic sulphur conversion within temperature range (30-45)^{o}C has been determined. The kinetic equation gave a satisfactory result and it is recommended that more frequent coal sampling e.g. every one or two days especially during the first 12 days of the process will provide a more accurate result.

The other direction in which this work should continue is towards the investigations of the application of this microbial coal desulphurisation to a larger scale. Although the results using the small reactor were promising much more study is required to determine the optimal conditions in large scale reactor.

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APPENDIX A:

GROWTH PHASES OF MICROORGANISMS

Growth phases of microorganisms can be divided to five stages/phases, there are:

1. **Lag and acceleration phases:**

Lag phase: a period of adaptation of inoculated cells to a novel environment. The number of live cells usually decreases. In this phase, a certain time elapses before the number of microorganisms begins to increase.

Acceleration phase: cells begin to multiply at an increasing rate.

2. **Exponential phase** (or logarithmic part of the growth curve):

The number of cells rises exponentially with time. In this phase the microorganisms are multiplying uniformly.

3. **Deceleration phase**: multiplication rate decreases.

4. **Stationary phase**: multiplication rate is in equilibrium with death rate.

5. **Death phase**: which may be subdivided into accelerated death phase, exponential death phase and death declination phase.

ad. 1. Lag and acceleration phases

When microorganisms are transferred into a fresh medium their concentration is often constant for some period (may be very short or last several hours). The terminology used by different authors is not consistent; the term lag is sometimes used for acceleration phase, in other cases it denotes a combination of lag phase (phase 1) and acceleration phase (phase 2). Both phases represent the period of preparation for multiplication. In the lag phase the microorganisms must obviously reorganize their metabolism in order to be able to perform rapid synthesis of substances characteristic for the phase of intensive multiplication. The first stage is immediately followed by the acceleration phase where the mass of single microbial cell grows considerably, generally their size increases and they are thus preparing for division which is associated with a great increase of enzymatic activity. This stage represents the beginning of multiplication. The length of these two phases is affected by the following factors:

a. Composition of previous medium in which the microorganisms grew before transfer into the new one.
b. Composition of new medium into which the microorganisms were transferred.
c. Type and age of the strain.

d. Number of cells.

e. Their genetical constitution.

f. Physical factors, e.g. temperature, pH, redox potential.

g. The physiological state of microorganisms during transfer.

Microorganisms inoculated from the stationary phase have long lag and acceleration phases. Microorganisms inoculated from the exponential phase usually grow with a lag phase. Cells in the acceleration are considered to be physiologically young, more sensitive to environmental changes, temperature, chemicals and more resistant to aggluration during electrophoresis than cells from later growth phases. The length of two phases may be defined as the time interval between the moment of inoculation and the moment when microbial growth has just assumed an exponential character. An undesirable extension of the lag phase causes economic losses.

• Characteristics:

Characteristics of both phases are as follows:

a. Growth rate exceeds multiplication rate and individual cells in this phase are bigger than in any other phase.

b. Microorganisms with a long generation time in the exponential phase exhibit also a longer acceleration phase. The poorer the medium the longer the acceleration phase. The phase is also extended on transferring the microorganisms into a medium with a composition different from that of the inoculum medium.

c. Other phases are usually unaffected by the lag and acceleration phases.

The duration of the lag and acceleration phases is minimized mainly by:

a. Using an actively growing culture from the exponential phase as inoculum.

b. Using medium of the same composition in cells stages of vegetative inoculum preparation (adaptation)

c. Using relatively large inoculum with a volume equal to (5-10)% of that of the following stage.

ad.2. <u>Exponential phase</u>

Microorganisms in this phase multiply at a uniform maximum specific rate and cells divide steadily at a constant rate. During this phase the microbial cell does not change its chemical composition and the replication of all cellular components proceeds consequently at the same ratio. The maximum multiplication rate is the result of a number of interdependent factors and biochemical and biophysical reactions and is maintained as long as the microorganisms have enough nutrients and the growth of the culture is not inhibited by the products of its own metabolism. During intensive growth

the essential nutrients for the formation of the cell mass and its division are rapidly utilized. This uniform multiplication continues therefore only as long as a sufficient amount of nutrients is available. The length of the period of uniform division of microorganisms therefore differs according to the available amount of essential nutrients. As microorganisms are often cultivated in a nutrient medium of empirical composition, multiplication can stop when the substrate is not yet completely exhausted, possibly only some components of the nutrients which are essential for the multiplication of the microorganisms are utilised and those become the limiting factor of multiplication. A nutrient of a certain composition forms the growth conditions for a limited amount of microorganisms so that the culture in a certain medium can attain only a certain maximum concentration. By the detailed knowledge of these relations and by supplying all the necessary nutrients as well as by the adjustment of other conditions (requirements of air, O_2, CO_2, optimum temperature) in these cultures a certain control of the amount of multiplying microorganisms and an increase in the productivity of growth is possible. The phase of exponential growth is the period of most rapid development of microorganisms. The culture is in the condition of balanced growth when all nutrients are present in excess and development is not inhibited by the products of metabolism.

ad.3. Deceleration phase

The loss of ability of the microorganisms to maintain the high specific growth rate achieved in the exponential phase and the growth phase (thus declines) is caused by the gradual exhaustion of nutrients and accumulation of toxic metabolic products. The growth curve in this phase has a variety of shapes, sometimes growth ceases immediately after energy source depletion, or it is relatively short and is followed by a prolonged deceleration phase. The duration of the deceleration phase depends on the method of the measurement. However, it is impossible to prevent the slowing down of microbial growth to such an extent that the amount of grown microorganisms is balanced by the number of dying cells. The concentration of nutrient decreases and the amount of products increases until finally the concentration limits are surpassed. Growth and dividing of cells slow down and continually retreat from the conditions of the exponential phase. The effective concentration of nutrients declines so low, that the specific growth rate gradually approaches zero.

ad.4. Stationary phase

This phase reflects the part of the growth curve in which the microbial population achieves a maximum size and for a certain period of time, also a constant cell size. The measurement of this apparent length of this phase depends on the methods of growth

measurement. In this phase cells often accumulate reserve substances (fats, starchs), in other cases the cells prepare for differentiation and start to sporulate. Its final mass and volume depend on the size of inoculum, on the starting concentration of nutrients and a combination of other factors together representing the sum of the conditions determining the course of the process. During the stationary phase the main metabolic pathways come to a standstill. The minor ones, however, can continue with gradual exhaustion of the nutrients and accumulation of the metabolites in the living system until a point is reached where exchange of free energy no longer occurs. The process stops and an equilibrium is established. The population remains constant for a time, perhaps as a result of complete cessation of division or the balancing of reproduction rate by an equivalent death rate.

ad.5. Death phase

The death of microorganisms is caused by chemical and physical factors but no general rule exists for the course of the death phase. Death may be slow or rapid and it may or may not connected with cell autolysis. Undoubtedly a variety of conditions contributes to microorganisms death, but the most important are the depletion of essential nutrients and the accumulation of inhibiting products. During the death phase, the number of viable cells decreases exponentially, essentially the inverse of growth during the logarithmic phase. Microorganisms die at different rates, just as they grow at different rates.

APPENDIX B

PYRITIC SULPHUR CONTENT BEFORE AND AFTER TREATMENT

• EXPERIMENTS WITH AN AIR FLOW OF 1.0 LITRE/MINUTE AND STIRRER SPEED OF 125 RPM

Experimental conditions	slurry conc. %w/v	% pyritic sulphur (before treatment)	% pyritic sulphur (after treatment) at 6th day	12 th	18th	24th
1.0 l/min.;	15	1.16	0.70	0.43	0.42	0.38
125 rpm;	20	1.16	0.74	0.55	0.31	0.30
30°C	25	1.16	0.63	0.48	0.21	0.21
	30	1.16	1.01	0.66	0.24	0.19
	35	1.16	1.01	0.93	0.55	0.33
1.0 l/min.;	15	1.13	0.78	0.20	0.06	0.06
125 rpm;	20	1.13	0.58	0.25	0.08	0.07
35°C	25(*)	1.13	1.13	n.a+	1.01	0.93
	25	1.13	0.49	0.13	0.07	0.00
	30	1.13	0.45	0.15	0.09	0.06
	35	1.13	0.58	0.51	0.30	0.18
1.0 l/min.;	15	1.12	0.42	0.20	0.17	0.06
125 rpm;	20	1.12	0.48	0.22	0.19	0.10
40°C	25	1.12	0.76	0.32	0.10	0.03
	30	1.12	0.65	0.27	0.09	0.05
	35	1.12	0.62	0.37	0.13	0.06
1.0 l/min.;	15	1.25	0.46	0.12	0.08	0.08
125 rpm;	20	1.25	0.62	0.47	0.46	0.21
45°C	25	1.25	0.84	0.21	0.12	0.05
	30	1.25	0.66	0.10	0.05	0.02
	35	1.25	0.77	0.49	0.18	0.03

(*) No culture, (+) Not analysed

APPENDIX B

PYRITIC SULPHUR CONTENT BEFORE AND AFTER TREATMENT

• EXPERIMENTS WITH AN AIR FLOW OF 1.5 LITRE/MINUTE AND STIRRER SPEED OF 125 RPM

Experimental conditions	slurry conc. %w/v	% pyritic sulphur (before treatment)	% pyritic sulphur (after treatment) at 6th day	12 th	18th	24th
1.5 l/min.;	15	1.07	0.54	0.23	0.18	0.10
125 rpm;	20	1.07	0.53	0.21	0.18	0.11
30°C	25	1.07	0.52	0.23	0.14	0.01
	30	1.07	0.67	0.24	0.08	0.07
	35	1.07	0.67	0.19	0.08	0.07
1.5 l/min.;	15	1.14	0.59	0.15	0.11	0.02
125 rpm;	20	1.14	0.71	0.18	0.11	0.05
35°C	25	1.13	0.44	0.11	0.07	0.05
	30	1.13	0.64	0.15	0.05	0.05
	35	1.13	0.42	0.45	0.07	0.07
1.5 l/min.;	15	1.29	0.30	0.06	0.06	0.05
125 rpm;	20	1.29	0.41	0.12	0.12	0.10
40°C	25	1.29	0.53	0.12	0.06	0.04
	30	1.29	0.53	0.12	0.07	0.05
	35	1.29	0.69	0.24	0.14	0.05
1.5 l/min.;	15	1.27	0.21	0.10	0.06	0.03
125 rpm;	20	1.27	0.81	0.40	0.35	0.19
45°C	25	1.27	0.59	0.23	0.08	0.06
	30	1.27	0.54	0.07	0.05	0.03
	35	1.27	0.53	0.06	0.06	0.04

APPENDIX B

PYRITIC SULPHUR CONTENT BEFORE AND AFTER TREATMENT

• EXPERIMENTS WITH AN AIR FLOW OF 1.5 LITRE/MINUTE AND STIRRER SPEED OF 175 RPM

Experimental conditions	slurry conc. %w/v	% pyritic sulphur (before treatment)	% pyritic sulphur (after treatment) at 6th day	12 th	18th	24th
1.5 l/min.;	15	1.03	0.67	0.18	0.10	0.07
175 rpm;	20	1.03	0.77	0.64	0.12	0.07
30°C	25	1.03	0.76	0.37	0.07	0.04
	30	1.03	0.92	0.26	0.12	0.07
	35	1.03	0.85	0.28	0.09	0.08
1.5 l/min.;	15	1.16	0.61	0.13	0.09	0.05
175 rpm;	20	1.16	0.68	0.16	0.12	0.04
35°C	25	1.16	0.67	0.17	0.09	0.09
	30	1.16	0.92	0.15	0.08	0.08
	35	1.16	0.88	0.25	0.13	0.07
1.5 l/min.;	15	1.37	0.46	0.17	0.09	0.08
175 rpm;	20	1.37	0.56	0.13	0.07	0.06
40°C	25	1.37	0.55	0.16	0.10	0.06
	30	1.37	0.68	0.25	0.11	0.05
	35	1.37	0.85	0.26	0.08	0.05
1.5 l/min.;	15	1.22	0.51	0.07	0.04	0.03
175 rpm;	20	1.22	0.43	0.07	0.07	0.05
45°C	25	1.22	0.46	0.19	0.10	0.07
	30	1.22	0.72	0.12	0.06	0.06
	35	1.22	0.73	0.09	0.03	0.03

APPENDIX C:

pH OF EXPERIMENTS

t day	15% pH I	20% pH I	25% pH I	30% pH I	35% pH I	15% pH II	20% pH II	25% pH II	30% pH II	35% pH II	15% pH III	20% pH III	25% pH III	30% pH III	35% pH III
Temperature of experiments: 30°Celsius. I. Experiment with air flow rate 1.0 litre/minute & stirrer rotation 125 rpm II. Experiment with air flow rate 1.5 litre/minute & stirrer rotation 125 rpm III. Experiment with air flow rate 1.5 litre/minute & stirrer rotation 175 rpm.															
1	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60
2	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.56	2.60	2.57	2.60	2.60	2.60	2.60	2.60
3	2.58	2.51	2.55	2.58	2.58	2.53	2.55	2.54	2.60	2.54	2.58	2.59	2.57	2.56	2.59
4	2.58	2.51	2.55	2.56	2.55	2.49	2.52	2.49	2.56	2.50	2.58	2.56	2.56	2.55	2.59
5	2.57	2.48	2.55	2.55	2.55	2.46	2.45	2.42	2.47	2.39	2.50	2.48	2.45	2.43	2.47
6	2.49	2.47	2.51	2.54	2.53	2.43	2.40	2.36	2.34	2.26	2.49	2.44	2.41	2.37	2.39
7	2.44	2.42	2.48	2.51	2.50	2.36	2.33	2.25	2.22	2.17	2.43	2.37	2.29	2.24	2.23
8	2.36	2.35	2.40	2.40	2.46	2.29	2.24	2.15	2.10	2.02	2.37	2.30	2.19	2.13	2.11
9	2.26	2.25	2.28	2.27	2.32	2.22	2.16	2.05	1.98	1.95	2.28	2.18	2.10	2.02	1.99
10	2.18	2.13	2.14	2.16	2.16	2.16	2.10	2.00	1.92	1.90	2.25	2.10	2.04	1.96	1.90
11	2.11	2.06	1.98	2.05	1.98	2.09	2.04	1.94	1.86	1.86	2.17	2.03	1.97	1.90	1.85
12	2.06	1.98	1.92	2.00	1.89	2.06	1.98	1.92	1.82	1.81	2.10	1.96	1.93	1.86	1.80
13	2.02	1.96	1.89	1.92	1.81	2.02	1.93	1.86	1.79	1.77	2.07	1.95	1.87	1.82	1.78
14	2.00	1.94	1.82	1.87	1.79	1.98	1.92	1.84	1.76	1.76	2.01	1.92	1.83	1.77	1.75
15	1.98	1.93	1.76	1.79	1.76	1.98	1.91	1.84	1.76	1.76	1.99	1.89	1.83	1.76	1.72
16	1.97	1.82	1.74	1.78	1.72	1.98	1.90	1.83	1.76	1.73	1.97	1.87	1.80	1.74	1.72
17	1.88	1.80	1.71	1.73	1.65	1.96	1.87	1.78	1.73	1.73	1.96	1.87	1.79	1.74	1.71
18	1.87	1.80	1.69	1.71	1.63	1.94	1.86	1.78	1.73	1.73	1.95	1.83	1.78	1.73	1.69
19	1.87	1.80	1.67	1.70	1.63	1.94	1.85	1.78	1.73	1.71	1.95	1.80	1.76	1.70	1.65
20	1.87	1.76	1.66	1.68	1.59	1.93	1.84	1.77	1.72	1.69	1.91	1.79	1.74	1.70	1.64
21	1.86	1.75	1.65	1.65	1.59	1.93	1.82	1.75	1.70	1.67	1.91	1.79	1.74	1.69	1.64
22	1.84	1.72	1.63	1.63	1.58	1.91	1.82	1.75	1.70	1.67	1.90	1.79	1.74	1.69	1.64
23	1.84	1.72	1.61	1.63	1.56	1.90	1.80	1.74	1.69	1.65	1.90	1.79	1.74	1.68	1.64
24	1.82	1.71	1.61	1.63	1.56	1.89	1.79	1.73	1.69	1.63	1.90	1.79	1.74	1.68	1.64
Temperature of experiments: 35°Celsius.															
1	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.56	2.60	2.64	2.60	2.60	2.60	2.60	2.60
2	2.57	2.53	2.55	2.55	2.57	2.60	2.56	2.50	2.60	2.60	2.57	2.59	2.55	2.58	2.60
3	2.48	2.43	2.43	2.47	2.47	2.57	2.51	2.45	2.57	2.60	2.53	2.54	2.51	2.56	2.58
4	2.41	2.33	2.31	2.41	2.41	2.49	2.43	2.33	2.46	2.54	2.50	2.50	2.50	2.54	2.55
5	2.39	2.29	2.11	2.18	2.19	2.39	2.32	2.18	2.30	2.48	2.39	2.35	2.31	2.43	2.52
6	2.37	2.22	2.03	2.06	2.13	2.28	2.19	2.04	2.12	2.36	2.30	2.21	2.07	2.20	2.41
7	2.29	2.19	1.93	1.95	2.06	2.18	2.11	1.93	1.97	2.25	2.19	2.07	1.99	2.02	2.13
8	2.17	2.07	1.89	1.87	2.02	2.14	2.04	1.84	1.89	2.16	2.07	1.94	1.87	1.88	1.94
9	2.06	2.03	1.87	1.82	1.98	2.07	2.00	1.80	1.86	2.15	2.00	1.87	1.78	1.79	1.81
10	2.04	1.98	1.85	1.80	1.96	1.98	1.90	1.76	1.78	2.07	1.93	1.80	1.74	1.74	1.72
11	1.98	1.94	1.83	1.79	1.94	1.89	1.85	1.69	1.72	1.98	1.90	1.78	1.72	1.72	1.69
12	1.96	1.92	1.82	1.78	1.92	1.87	1.80	1.69	1.69	1.89	1.84	1.74	1.70	1.67	1.66
13	1.96	1.89	1.82	1.78	1.89	1.86	1.78	1.67	1.67	1.84	1.84	1.73	1.67	1.66	1.63
14	1.93	1.88	1.80	1.76	1.87	1.86	1.76	1.64	1.66	1.75	1.84	1.71	1.65	1.65	1.61
15	1.87	1.88	1.78	1.74	1.84	1.83	1.74	1.64	1.64	1.69	1.84	1.70	1.65	1.64	1.59
16	1.83	1.84	1.75	1.74	1.82	1.83	1.71	1.64	1.64	1.68	1.82	1.70	1.62	1.61	1.58
17	1.83	1.84	1.72	1.72	1.78	1.81	1.70	1.63	1.61	1.66	1.82	1.68	1.61	1.59	1.58
18	1.83	1.80	1.68	1.70	1.78	1.80	1.70	1.62	1.60	1.65	1.79	1.66	1.61	1.59	1.58
19	1.80	1.77	1.65	1.66	1.75	1.79	1.70	1.60	1.59	1.63	1.79	1.66	1.61	1.59	1.55
20	1.76	1.73	1.63	1.66	1.72	1.79	1.68	1.59	1.59	1.62	1.79	1.66	1.61	1.59	1.54

21	1.74	1.71	1.62	1.64	1.72	1.78	1.68	1.59	1.59	1.62	1.79	1.66	1.60	1.58	1.53
22	1.72	1.71	1.58	1.64	1.70	1.78	1.68	1.59	1.57	1.61	1.79	1.66	1.60	1.57	1.53
23	1.72	1.68	1.57	1.62	1.68	1.78	1.68	1.58	1.57	1.59	1.79	1.66	1.60	1.57	1.53
24	1.72	1.68	1.57	1.62	1.68	1.77	1.67	1.58	1.56	1.59	1.79	1.66	1.60	1.57	1.53
Temperature of experiments: 40°Celsius.															
1	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60
2	2.60	2.60	2.59	2.59	2.59	2.56	2.60	2.57	2.60	2.60	2.60	2.59	2.58	2.57	2.60
3	2.58	2.55	2.52	2.57	2.57	2.50	2.57	2.55	2.58	2.59	2.55	2.58	2.58	2.56	2.60
4	2.47	2.48	2.50	2.45	2.46	2.27	2.43	2.33	2.32	2.41	2.45	2.44	2.46	2.42	2.45
5	2.41	2.44	2.39	2.40	2.44	2.06	2.21	2.06	2.02	2.13	2.23	2.17	2.17	2.12	2.12
6	2.38	2.37	2.29	2.32	2.37	1.95	2.03	1.98	1.84	1.93	2.06	1.97	1.92	1.86	1.87
7	2.09	2.08	2.02	2.07	2.06	1.91	1.95	1.85	1.79	1.81	1.94	1.87	1.81	1.74	1.77
8	2.01	1.98	1.91	1.99	1.99	1.85	1.88	1.76	1.70	1.70	1.88	1.83	1.75	1.69	1.73
9	1.97	1.87	1.82	1.87	1.90	1.82	1.82	1.70	1.65	1.67	1.83	1.76	1.68	1.64	1.64
10	1.91	1.82	1.79	1.84	1.88	1.82	1.78	1.67	1.62	1.62	1.78	1.72	1.68	1.62	1.58
11	1.88	1.82	1.78	1.83	1.82	1.79	1.76	1.66	1.62	1.61	1.78	1.72	1.65	1.61	1.58
12	1.88	1.78	1.76	1.78	1.74	1.79	1.72	1.66	1.61	1.61	1.71	1.69	1.62	1.53	1.52
13	1.79	1.67	1.65	1.64	1.67	1.74	1.72	1.61	1.57	1.59	1.71	1.66	1.61	1.53	1.51
14	1.76	1.67	1.62	1.61	1.64	1.74	1.72	1.61	1.56	1.56	1.71	1.61	1.57	1.51	1.48
15	1.73	1.64	1.57	1.57	1.59	1.74	1.70	1.59	1.54	1.54	1.71	1.61	1.57	1.51	1.48
16	1.72	1.61	1.52	1.52	1.54	1.74	1.70	1.59	1.54	1.54	1.71	1.61	1.57	1.51	1.47
17	1.72	1.59	1.49	1.44	1.49	1.70	1.68	1.57	1.51	1.52	1.68	1.61	1.57	1.51	1.47
18	1.72	1.55	1.48	1.43	1.48	1.70	1.66	1.57	1.51	1.52	1.68	1.58	1.57	1.51	1.47
19	1.64	1.52	1.45	1.40	1.44	1.70	1.66	1.57	1.51	1.52	1.68	1.58	1.57	1.51	1.46
20	1.60	1.50	1.41	1.40	1.44	1.70	1.66	1.55	1.49	1.50	1.68	1.56	1.53	1.46	1.43
21	1.60	1.48	1.41	1.38	1.42	1.67	1.63	1.53	1.47	1.48	1.66	1.55	1.50	1.45	1.43
22	1.54	1.46	1.36	1.38	1.37	1.67	1.63	1.51	1.46	1.48	1.66	1.55	1.50	1.45	1.43
23	1.54	1.46	1.36	1.29	1.33	1.67	1.63	1.51	1.46	1.48	1.65	1.53	1.50	1.45	1.42
24	1.54	1.46	1.36	1.29	1.33	1.67	1.63	1.51	1.46	1.48	1.65	1.53	1.50	1.45	1.42
Temperature of experiments: 45°Celsius.															
1	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60	2.60
2	2.54	2.60	2.60	2.60	2.60	2.57	2.60	2.60	2.60	2.60	2.57	2.60	2.57	2.60	2.60
3	2.44	2.48	2.41	2.39	2.47	2.38	2.50	2.40	2.44	2.50	2.54	2.58	2.54	2.53	2.53
4	2.37	2.39	2.30	2.21	2.28	2.02	2.31	2.36	2.08	2.24	2.32	2.37	2.32	2.38	2.39
5	2.20	2.37	2.18	1.99	2.10	1.82	2.21	2.04	1.85	1.98	2.05	2.09	1.98	2.11	2.11
6	2.15	2.30	2.11	1.94	2.03	1.79	2.13	1.83	1.74	1.85	1.84	1.88	1.73	1.87	1.95
7	1.85	2.27	1.99	1.85	1.93	1.72	2.05	1.74	1.64	1.75	1.79	1.76	1.71	1.73	1.70
8	1.80	2.16	1.83	1.80	1.80	1.70	1.97	1.65	1.55	1.66	1.74	1.68	1.60	1.66	1.59
9	1.78	2.09	1.67	1.71	1.67	1.67	1.89	1.60	1.52	1.57	1.65	1.59	1.56	1.56	1.53
10	1.73	2.07	1.62	1.61	1.57	1.66	1.88	1.58	1.50	1.55	1.63	1.57	1.52	1.53	1.48
11	1.71	2.02	1.59	1.55	1.57	1.61	1.83	1.54	1.49	1.48	1.61	1.55	1.51	1.50	1.47
12	1.68	1.98	1.57	1.48	1.53	1.61	1.82	1.50	1.42	1.44	1.61	1.55	1.51	1.50	1.44
13	1.68	1.96	1.57	1.48	1.47	1.59	1.78	1.50	1.42	1.44	1.60	1.55	1.46	1.47	1.44
14	1.68	1.96	1.55	1.48	1.47	1.57	1.77	1.49	1.40	1.43	1.60	1.54	1.46	1.47	1.42
15	1.66	1.96	1.55	1.45	1.44	1.56	1.76	1.46	1.36	1.39	1.59	1.53	1.46	1.45	1.42
16	1.66	1.94	1.52	1.45	1.43	1.56	1.76	1.46	1.36	1.39	1.59	1.51	1.46	1.44	1.39
17	1.66	1.94	1.52	1.44	1.43	1.56	1.76	1.46	1.36	1.39	1.59	1.48	1.43	1.42	1.39
18	1.66	1.92	1.49	1.44	1.43	1.56	1.76	1.44	1.36	1.38	1.58	1.48	1.43	1.41	1.39
19	1.60	1.92	1.49	1.44	1.39	1.56	1.76	1.43	1.35	1.38	1.58	1.48	1.43	1.41	1.39
20	1.60	1.90	1.49	1.43	1.38	1.56	1.76	1.43	1.35	1.36	1.58	1.48	1.42	1.40	1.39
21	1.59	1.88	1.46	1.43	1.38	1.56	1.75	1.42	1.35	1.35	1.58	1.48	1.39	1.38	1.39
22	1.59	1.88	1.46	1.40	1.36	1.56	1.75	1.42	1.35	1.35	1.58	1.48	1.39	1.38	1.37
23	1.59	1.88	1.46	1.40	1.36	1.55	1.75	1.42	1.34	1.33	1.55	1.48	1.38	1.37	1.37
24	1.59	1.88	1.46	1.40	1.36	1.55	1.75	1.42	1.34	1.33	1.55	1.48	1.38	1.37	1.37

APPENDIX D

AMOUNTS OF PYRITIC SULPHUR REMOVAL AND SULPHURIC ACID PRODUCED

Condition	Day	Pyrite removal (mM/l)	H_2SO_4 produced (mM/l)
15%w/v	6	5.31	1.62
30°C	12	8.43	4.35
1.0l/min.	18	8.54	6.74
125 rpm	24	9.00	7.57
20%w/v	6	6.46	1.69
30°C	12	9.39	5.24
1.0l/min.	18	13.08	7.92
125 rpm	24	13.23	9.75
25%w/v	6	10.19	1.55
30°C	12	13.08	6.01
1.0l/min.	18	18.27	10.21
125 rpm	24	18.27	12.27
30%w/v	6	3.46	1.44
30°C	12	11.54	5.00
1.0l/min.	18	21.24	9.75
125 rpm	24	22.39	11.72
35%w/v	6	4.04	1.48
30°C	12	6.19	6.44
1.0l/min.	18	16.43	11.72
125 rpm	24	22.35	14.42
15%w/v	6	4.04	2.13
35°C	12	10.73	5.48
1.0l/min.	18	12.35	7.40
125 rpm	24	12.35	9.53
20%w/v	6	8.46	3.01
35°C	12	13.54	6.01
1.0l/min.	18	16.16	7.92
125 rpm	24	16.31	10.45
25%w/v	6	12.31	4.67
35°C	12	19.24	7.57
1.0l/min.	18	20.39	10.45
125 rpm	24	21.74	13.46
30%w/v	6	15.70	4.35
35°C	12	22.62	8.30
1.0l/min.	18	24.01	9.98
125 rpm	24	24.70	11.99
35%w/v	6	14.81	3.71
35°C	12	16.70	6.01
1.0l/min.	18	22.35	8.30
125 rpm	24	25.58	10.45
15%w/v	6	8.66	2.08
40°C	12	11.31	6.59
1.0l/min.	18	11.77	9.53
125 rpm	24	13.04	14.42
20%w/v	6	10.46	2.13
40°C	12	14.77	8.30
1.0l/min.	18	15.39	14.09
125 rpm	24	16.77	17.34

25%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	7.31 16.54 20.97 22.51	2.56 8.69 16.56 21.83
30%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	11.54 21.01 25.39 26.55	2.39 8.30 18.58 25.64
35%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	14.27 21.54 28.55 30.70	2.13 9.10 16.56 23.87
15%w/v 45°C 1.0l/min. 125 rpm	6 12 18 24	7.96 13.04 13.50 13.50	3.54 10.45 10.94 12.85
20%w/v 45°C 1.0l/min. 125 rpm	6 12 18 24	9.69 10.46 12.16 16.00	2.51 5.24 6.01 6.59
25%w/v 45°C 1.0l/min. 125 rpm	6 12 18 24	7.89 20.01 21.74 23.08	3.88 13.46 16.18 17.34
30%w/v 45°C 1.0l/min. 125 rpm	6 12 18 24	13.62 26.55 27.70 28.39	5.74 16.56 18.15 19.91
35%w/v 45°C 1.0l/min. 125 rpm	6 12 18 24	12.93 20.47 28.81 32.85	4.67 14.76 18.58 21.83
15%w/v 45°C 1.5l/min. 125 rpm	6 12 18 24	12.23 13.50 13.97 14.31	8.11 12.27 13.77 14.09
20%w/v 45°C 1.5l/min. 125 rpm	6 12 18 24	7.08 13.39 14.16 16.62	3.71 7.57 8.69 8.89
25%w/v 45°C 1.5l/min. 125 rpm	6 12 18 24	13.08 22.51 22.89 23.28	7.40 15.81 18.15 19.01
30%w/v 45°C 1.5l/min. 125 rpm	6 12 18 24	16.85 27.70 28.16 28.62	9.10 19.01 21.83 22.85
35%w/v 45°C 1.5l/min. 125 rpm	6 12 18 24	19.93 32.59 32.59 33.12	7.06 18.15 20.84 23.39
15%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	11.43 14.20 14.20 14.31	5.61 8.11 9.98 10.69

20%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	13.54 18.00 18.00 18.31	4.67 9.53 10.94 11.72
25%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	14.62 22.51 23.66 24.04	5.24 10.94 13.46 15.45
30%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	16.62 27.01 28.16 28.62	7.23 12.27 15.45 17.34
35%w/v 40°C 1.0l/min. 125 rpm	6 12 18 24	16.16 28.28 30.97 33.39	5.87 12.27 15.10 16.56
15%w/v 40°C 1.5l/min. 175 rpm	6 12 18 24	10.50 13.85 14.77 14.89	4.35 9.75 10.45 11.19
20%w/v 40°C 1.5l/min. 175 rpm	6 12 18 24	12.46 19.08 20.01 20.16	5.36 10.21 13.15 14.76
25%w/v 40°C 1.5l/min. 175 rpm	6 12 18 24	15.77 23.28 24.43 25.20	6.01 11.99 13.46 15.81
30%w/v 40°C 1.5l/min. 175 rpm	6 12 18 24	13.62 25.85 29.08 30.47	6.90 14.76 15.45 17.74
35%w/v 40°C 1.5l/min. 175 rpm	6 12 18 24	14.00 29.89 34.74 35.55	6.74 15.10 16.94 19.01
15%w/v 45°C 1.5l/min. 175 rpm	6 12 18 24	8.19 13.27 13.62 13.73	7.23 12.27 13.15 14.09
20%w/v 45°C 1.5l/min. 175 rpm	6 12 18 24	12.16 17.70 17.70 18.00	6.59 14.09 16.56 16.56
25%w/v 45°C 1.5l/min. 175 rpm	6 12 18 24	14.62 19.81 21.54 22.12	9.31 15.45 18.58 20.84
30%w/v 45°C 1.5l/min. 175 rpm	6 12 18 24	11.54 25.39 26.78 26.78	6.74 15.81 19.45 21.33
35%w/v 45°C 1.5l/min. 175 rpm	6 12 18 24	13.20 30.43 32.05 32.05	5.61 18.15 20.37 21.33

15%w/v 35°C 1.5l/min. 175 rpm	6 12 18 24	6.35 11.89 12.35 12.81	2.51 7.23 8.11 8.11
20%w/v 35°C 1.5l/min. 175 rpm	6 12 18 24	7.39 15.39 16.00 17.24	3.09 9.10 10.94 10.94
25%w/v 35°C 1.5l/min. 175 rpm	6 12 18 24	9.43 19.04 20.58 20.58	4.26 9.98 12.27 12.56
30%w/v 35°C 1.5l/min. 175 rpm	6 12 18 24	5.54 23.31 24.93 24.93	3.15 10.69 12.85 13.46
35%w/v 35°C 1.5l/min. 175 rpm	6 12 18 24	7.54 24.51 27.74 29.35	1.95 10.94 13.15 14.76
15%w/v 30°C 1.5l/min. 175 rpm	6 12 18 24	4.15 9.81 10.73 11.08	1.62 3.97 5.61 6.29
20%w/v 30°C 1.5l/min. 175 rpm	6 12 18 24	4.00 6.00 14.00 14.77	1.82 5.48 7.40 8.11
25%w/v 30°C 1.5l/min. 175 rpm	6 12 18 24	5.19 12.70 18.47 19.04	1.95 5.87 8.30 9.10
30%w/v 30°C 1.5l/min. 175 rpm	6 12 18 24	2.54 17.77 21.01 22.16	2.13 6.90 9.31 10.45
35%w/v 30°C 1.5l/min. 175 rpm	6 12 18 24	4.85 20.20 25.31 25.58	2.04 7.92 10.21 11.45
15%w/v 30°C 1.5l/min. 125 rpm	6 12 18 24	6.12 9.69 10.27 11.20	1.86 4.35 5.74 6.44
20%w/v 30°C 1.5l/min. 125 rpm	6 12 18 24	8.31 13.23 13.70 14.77	1.99 5.24 6.90 8.11
25%w/v 30°C 1.5l/min. 125 rpm	6 12 18 24	7.50 16.73 18.85 19.04	2.18 6.01 8.30 9.31
30%w/v 30°C 1.5l/min. 125 rpm	6 12 18 24	9.23 19.16 22.85 23.08	2.29 7.57 9.31 10.21

35%w/v 30°C 1.5l/min. 125 rpm	6 12 18 24	10.77 23.70 26.66 26.93	2.75 7.74 9.31 11.72
15%w/v 35°C 1.5l/min. 125 rpm	6 12 18 24	6.35 11.43 11.89 12.93	2.62 6.74 7.92 8.49
20%w/v 35°C 1.5l/min. 125 rpm	6 12 18 24	6.62 14.77 15.85 16.77	3.23 7.92 9.98 10.69
25%w/v 35°C 1.5l/min. 125 rpm	6 12 18 24	13.46 19.81 20.58 20.97	4.56 10.21 11.99 13.15
30%w/v 35°C 1.5l/min. 125 rpm	6 12 18 24	11.54 22.85 25.16 25.16	3.79 10.21 12.56 13.77
35%w/v 35°C 1.5l/min. 125 rpm	6 12 18 24	11.31 18.58 28.81 28.81	2.18 6.44 11.12 12.85

APPENDIX E:

COMPARISON OF PREDICTED AND EXPERIMENTAL C_t/C_o

Exp. C_t/C_o 1.5l/min 175 rpm	C_t/C_o calc.	Δ C_t/C_o	%Δ C_t/C_o	Exp. C_t/C_o 1.5l/min 125 rpm	C_t/C_o calc	Δ C_t/C_o	%Δ C_t/C_o	Exp. C_t/C_o 1.0l/min 125 rpm	C_t/C_o calc.	Δ C_t/C_o	%Δ C_t/C_o
1.00	0.95	-0.05	-5.45	1.00	0.95	-0.05	-4.65	1.00	0.84	-0.16	-15.97
0.53	0.61	0.09	8.73	0.52	0.59	0.08	7.73	0.60	0.76	0.16	15.86
0.11	0.10	-0.01	-0.86	0.13	0.14	0.00	0.35	0.37	0.46	0.09	8.53
0.08	0.05	-0.03	-2.95	0.10	0.06	-0.04	-3.99	0.36	0.32	-0.04	-4.14
0.04	0.05	0.01	0.50	0.02	0.02	0.01	0.54	0.33	0.29	-0.04	-4.25
1.00	0.99	-0.01	-1.12	1.00	1.02	0.02	1.52	1.00	0.88	-0.12	-11.83
0.59	0.60	0.02	1.86	0.62	0.59	-0.03	-3.32	0.64	0.79	0.15	14.94
0.14	0.14	0.00	0.41	0.16	0.18	0.03	2.70	0.47	0.43	-0.04	-4.24
0.10	0.06	-0.04	-4.02	0.10	0.08	-0.02	-1.54	0.27	0.30	0.03	3.38
0.03	0.06	0.03	2.88	0.04	0.05	0.01	0.61	0.26	0.24	-0.02	-2.29
1.00	1.02	0.02	2.37	1.00	0.97	-0.03	-2.75	1.00	0.82	-0.18	-18.30
0.58	0.52	-0.06	-5.59	0.39	0.44	0.06	5.80	0.54	0.76	0.21	21.43
0.15	0.17	0.02	2.47	0.10	0.11	0.02	1.72	0.41	0.37	-0.05	-4.66
0.08	0.09	0.01	0.85	0.06	0.05	-0.01	-1.38	0.18	0.22	0.03	3.41
0.08	0.08	-0.00	-0.10	0.04	0.01	-0.03	-3.40	0.18	0.16	-0.02	-1.88
1.00	1.07	0.07	6.66	1.00	1.00	0.00	0.36	1.00	0.98	-0.02	-2.35
0.79	0.68	-0.11	-11.48	0.56	0.55	-0.01	-1.09	0.87	0.93	0.06	5.66
0.13	0.16	0.03	3.41	0.13	0.14	0.01	1.31	0.57	0.48	-0.09	-8.58
0.07	0.09	0.02	1.68	0.04	0.06	0.02	1.59	0.21	0.24	0.04	3.77
0.07	0.07	-0.00	-0.26	0.04	0.02	-0.02	-2.18	0.16	0.18	0.01	1.50
1.00	0.96	-0.04	-3.56	1.00	0.94	-0.06	-5.60	1.00	0.99	-0.01	-1.05
0.76	0.81	0.05	4.79	0.63	0.73	0.10	10.04	0.87	0.95	0.08	8.11
0.22	0.18	-0.03	-3.20	0.39	0.32	-0.08	-7.80	0.80	0.61	-0.20	-19.54
0.11	0.12	0.00	0.49	0.06	0.10	0.04	4.32	0.47	0.47	-0.01	-0.81
0.06	0.08	0.02	1.51	0.06	0.05	-0.01	-0.98	0.28	0.42	0.13	13.30
1.00	0.89	-0.11	-10.93	1.00	0.86	-0.14	-13.51	1.00	0.97	-0.03	-3.35
0.65	0.75	0.10	10.19	0.50	0.67	0.17	16.87	0.69	0.71	0.02	1.53
0.17	0.26	0.09	8.75	0.22	0.26	0.04	4.15	0.18	0.24	0.06	6.35
0.10	0.07	-0.02	-2.33	0.17	0.12	-0.05	-4.70	0.05	0.09	0.04	3.99
0.07	0.01	-0.06	-5.70	0.09	0.06	-0.03	-2.85	0.05	-0.03	-0.08	-8.49
1.00	0.99	-0.01	-1.16	1.00	0.86	-0.14	-13.62	1.00	0.96	-0.04	-3.85
0.75	0.83	0.08	8.02	0.50	0.67	0.17	16.96	0.51	0.56	0.05	4.63
0.62	0.35	-0.28	-27.53	0.20	0.22	0.03	2.53	0.22	0.24	0.02	2.11
0.12	0.22	0.10	9.90	0.17	0.13	-0.04	-4.05	0.07	0.12	0.04	4.46
0.07	0.18	0.11	10.74	0.10	0.06	-0.04	-4.43	0.06	-0.01	-0.07	-7.35
1.00	0.98	-0.02	-1.53	1.00	0.95	-0.05	-5.17	1.00	0.99	-0.01	-1.14
0.74	0.78	0.04	4.34	0.64	0.69	0.06	5.90	0.43	0.41	-0.02	-2.07
0.36	0.27	-0.09	-9.19	0.19	0.23	0.04	4.23	0.12	0.20	0.09	8.58
0.07	0.11	0.04	3.88	0.08	0.08	-0.00	-0.29	0.06	0.06	-0.00	-0.25
0.04	0.06	0.03	2.51	0.07	0.03	-0.05	-4.65	0.00	-0.05	-0.05	-5.14
1.00	1.06	0.06	5.84	1.00	0.98	-0.02	-1.62	1.00	0.98	-0.02	-2.38
0.89	0.81	-0.08	-8.14	0.63	0.65	0.02	2.24	0.40	0.44	0.04	4.17
0.25	0.26	0.01	1.19	0.22	0.20	-0.02	-1.94	0.13	0.16	0.03	2.92
0.12	0.12	0.01	0.82	0.07	0.13	0.05	5.13	0.08	0.08	0.00	0.28
0.07	0.07	0.00	0.31	0.07	0.03	-0.04	-3.80	0.05	0.00	-0.05	-5.01
1.00	1.02	0.02	1.60	1.00	0.98	-0.02	-1.75	1.00	0.99	-0.01	-1.27
0.83	0.81	-0.01	-1.47	0.63	0.64	0.02	1.65	0.51	0.57	0.06	5.97
0.27	0.23	-0.04	-3.85	0.18	0.19	0.02	1.53	0.45	0.39	-0.06	-6.34
0.09	0.13	0.04	3.83	0.07	0.11	0.04	3.82	0.27	0.26	-0.00	-0.10

0.08	0.08	-0.00	-0.09	0.07	0.01	-0.05	-5.25	0.16	0.18	0.02	1.70
1.00	1.03	0.03	2.67	1.00	0.99	-0.01	-1.45	1.00	0.79	-0.21	-21.12
0.42	0.31	-0.11	-10.78	0.17	0.23	0.07	6.80	0.38	0.62	0.25	24.75
0.06	0.09	0.04	3.61	0.08	0.07	-0.01	-1.26	0.18	0.24	0.06	6.12
0.03	0.07	0.03	3.24	0.05	0.02	-0.03	-2.75	0.15	0.12	-0.03	-2.65
0.02	0.04	0.01	1.23	0.02	0.01	-0.01	-1.32	0.06	-0.01	-0.07	-7.09
1.00	0.99	-0.01	-0.90	1.00	1.03	0.03	2.52	1.00	0.81	-0.19	-19.14
0.35	0.37	0.02	1.96	0.64	0.59	-0.05	-5.19	0.43	0.66	0.22	22.26
0.06	0.09	0.03	3.11	0.31	0.30	-0.02	-1.88	0.20	0.26	0.06	6.41
0.06	0.03	-0.03	-2.91	0.28	0.24	-0.04	-3.55	0.17	0.11	-0.06	-5.53
0.04	0.03	-0.01	-1.27	0.15	0.23	0.08	8.11	0.09	0.05	-0.04	-4.01
1.00	1.01	0.01	1.06	1.00	1.02	0.02	2.32	1.00	0.96	-0.04	-3.81
0.38	0.33	-0.04	-4.26	0.46	0.39	-0.07	-7.46	0.68	0.72	0.04	3.91
0.16	0.16	0.01	0.77	0.08	0.12	0.04	3.98	0.28	0.31	0.03	2.98
0.08	0.10	0.02	1.93	0.06	0.07	0.01	0.63	0.09	0.08	-0.01	-1.01
0.06	0.06	0.00	0.50	0.05	0.05	0.01	0.56	0.03	0.00	-0.02	-2.07
1.00	1.05	0.05	4.80	1.00	1.02	0.02	2.23	1.00	0.89	-0.11	-11.33
0.59	0.45	-0.14	-13.53	0.43	0.35	-0.08	-7.88	0.58	0.69	0.11	10.92
0.10	0.15	0.06	5.58	0.06	0.09	0.04	3.97	0.24	0.32	0.08	7.63
0.05	0.08	0.03	3.19	0.04	0.05	0.01	0.83	0.08	0.08	-0.01	-0.82
0.05	0.05	-0.00	-0.06	0.02	0.03	0.01	0.83	0.04	-0.02	-0.06	-6.36
1.00	1.04	0.04	4.12	1.00	1.00	-0.00	-0.07	1.00	0.87	-0.13	-12.54
0.60	0.51	-0.09	-9.01	0.42	0.41	-0.01	-0.66	0.56	0.72	0.16	16.33
0.07	0.09	0.02	1.64	0.05	0.09	0.04	4.17	0.33	0.30	-0.03	-3.08
0.02	0.05	0.02	2.45	0.05	0.04	-0.01	-0.54	0.12	0.13	0.01	1.29
0.02	0.03	0.01	0.81	0.03	0.00	-0.03	-2.89	0.05	0.03	-0.02	-2.02
1.00	0.96	-0.04	-4.02	1.00	0.98	-0.02	-2.18	1.00	0.95	-0.05	-4.83
0.34	0.43	0.10	9.80	0.23	0.28	0.05	4.89	0.45	0.53	0.09	8.53
0.12	0.09	-0.03	-3.14	0.05	0.11	0.06	6.34	0.10	0.10	0.00	0.04
0.07	0.06	-0.00	-0.22	0.05	0.01	-0.03	-3.30	0.06	0.08	0.01	1.38
0.06	0.03	-0.02	-2.41	0.04	-0.02	-0.06	-5.74	0.06	0.01	-0.05	-5.12
1.00	0.99	-0.01	-0.80	1.00	0.96	-0.04	-4.09	1.00	0.93	-0.07	-7.15
0.41	0.41	-0.00	-0.02	0.32	0.41	0.10	9.60	0.50	0.65	0.15	15.38
0.09	0.15	0.05	5.43	0.09	0.12	0.02	2.42	0.46	0.35	-0.10	-10.33
0.05	0.05	-0.00	-0.38	0.09	0.06	-0.03	-3.32	0.37	0.30	-0.07	-7.10
0.04	0.00	-0.04	-4.28	0.08	0.03	-0.05	-4.64	0.17	0.26	0.09	9.18
1.00	1.00	-0.00	-0.03	1.00	0.99	-0.01	-1.01	1.00	1.03	0.03	3.14
0.40	0.40	-0.01	-0.53	0.41	0.42	0.01	1.16	0.67	0.62	-0.05	-5.41
0.12	0.13	0.01	1.31	0.09	0.13	0.04	3.65	0.17	0.16	-0.01	-0.57
0.07	0.09	0.01	1.26	0.05	0.05	0.00	0.07	0.10	0.09	-0.00	-0.12
0.04	0.02	-0.02	-2.04	0.03	-0.01	-0.04	-3.88	0.04	0.07	0.03	2.95
1.00	1.05	0.05	4.76	1.00	1.02	0.02	1.93	1.00	1.02	0.02	2.36
0.57	0.43	-0.14	-13.75	0.44	0.36	-0.08	-8.49	0.53	0.47	-0.06	-5.51
0.18	0.16	-0.03	-2.51	0.09	0.16	0.06	6.35	0.08	0.09	0.01	0.92
0.08	0.14	0.06	6.05	0.05	0.07	0.02	1.51	0.04	0.06	0.02	1.58
0.04	0.09	0.05	5.44	0.04	0.03	-0.01	-1.29	0.02	0.02	0.01	0.64
1.00	1.06	0.06	6.04	1.00	1.03	0.03	2.95	1.00	1.02	0.02	2.20
0.62	0.46	-0.16	-16.20	0.53	0.46	-0.07	-7.46	0.62	0.61	-0.01	-0.69
0.19	0.17	-0.02	-1.99	0.19	0.19	0.00	0.23	0.39	0.25	-0.15	-14.51
0.06	0.13	0.07	7.03	0.11	0.11	0.00	0.34	0.14	0.17	0.03	3.05
0.04	0.09	0.05	5.10	0.04	0.08	0.04	3.92	0.02	0.12	0.10	9.98

APPENDIX F:

CALCULATION OF C_t/C_o VALUES (BASED ON pH EQUATION)

pH_t/pH_o	C_t/C_o	C_t/C_o calc.	$\Delta C_t/C_o$	% $\Delta C_t/C_o$	%w/v no.exp.
1	1	0.8403	-0.1597	-15.97	**15% w/v**
0.95769231	0.60334429	0.76199269	0.15864841	15.8648406	**exp.1**
0.79230769	0.37062136	0.45588231	0.08526095	8.52609485	
0.71923077	0.36200149	0.32062423	-0.0413773	-4.1377263	
0.7	0.32752427	0.28503	-0.0424943	-4.2494272	
1	1	0.8817	-0.1183	-11.83	**20% w/v**
0.95	0.63795294	0.78735	0.14939706	14.9397059	**exp.1**
0.76153846	0.47415462	0.43172308	-0.0424315	-4.2431545	
0.69230769	0.26725098	0.30108462	0.03383363	3.3833635	
0.65769231	0.25862969	0.23576538	-0.0228643	-2.2864307	
1	1	0.817	-0.183	-18.3	**25% w/v**
0.96538462	0.54318288	0.75747538	0.21429251	21.4292507	**exp.1**
0.73846154	0.41385388	0.36725846	-0.0465954	-4.6595416	
0.65	0.18106096	0.21514	0.03407904	3.40790408	
0.61923077	0.18106096	0.16222923	-0.0188317	-1.8831728	
1	1	0.9765	-0.0235	-2.35	**30% w/v**
0.97692308	0.87055713	0.92715462	0.05659748	5.65974832	**exp.1**
0.76923077	0.56887901	0.48304615	-0.0858329	-8.583286	
0.65769231	0.2068652	0.24454346	0.03767826	3.76782636	
0.62692308	0.16376848	0.17874962	0.01498113	1.49811314	
1	1	0.9895	-0.0105	-1.05	**35% w/v**
0.97307692	0.87065013	0.95172154	0.08107141	8.10714104	**exp.1**
0.72692308	0.80168758	0.60631846	-0.1953691	-19.536912	
0.62692308	0.47411652	0.46599846	-0.0081181	-0.8118056	
0.59230769	0.28446991	0.41742615	0.13295624	13.2956243	
1	1	0.9665	-0.0335	-3.35	**15% w/v**
0.91153846	0.69022469	0.70553846	0.01531377	1.53137683	**exp.2**
0.75384615	0.17688267	0.24034615	0.06346349	6.34634851	
0.70384615	0.05297239	0.09284615	0.03987376	3.98737612	
0.66153846	0.05297239	-0.0319615	-0.0849339	-8.4933931	
1	1	0.9615	-0.0385	-3.85	**20% w/v**
0.85384615	0.51330362	0.55962077	0.04631715	4.63171465	**exp.2**
0.73846154	0.22128522	0.24234769	0.02106247	2.10624709	
0.69230769	0.07085164	0.11543846	0.04458682	4.45868227	
0.64615385	0.06200288	-0.0114708	-0.0734736	-7.3473644	
1	1	0.9886	-0.0114	-1.14	**25% w/v**
0.78076923	0.43372723	0.41305346	-0.0206738	-2.0673769	**exp.2**
0.7	0.11519871	0.20101	0.08581129	8.58112879	
0.64615385	0.06211086	0.05964769	-0.0024632	-0.2463163	
0.60384615	1.7479E-05	-0.0514227	-0.0514402	-5.1440172	

1	1	0.9762	-0.0238	-2.38	**30% w/v**
0.79230769	0.39815069	0.43987615	0.04172546	4.17254637	**exp.2**
0.68461538	0.13262883	0.16178231	0.02915347	2.91534733	
0.65384615	0.07952454	0.08232692	0.00280238	0.28023832	
0.62307692	0.05297239	0.00287154	-0.0501009	-5.0100854	
1	1	0.9873	-0.0127	-1.27	**35% w/v**
0.81923077	0.51326323	0.572995	0.05973177	5.97317729	**exp.2**
0.73846154	0.45131515	0.38788	-0.0634351	-6.343515	
0.68461538	0.26547026	0.26447	-0.0010003	-0.100026	
0.64615385	0.15927309	0.17632	0.01704691	1.70469142	
1	1	0.7888	-0.2112	-21.12	**15% w/v**
0.91538462	0.37501733	0.62248846	0.24747113	24.7471133	**exp.3**
0.72307692	0.18335596	0.24450769	0.06115174	6.11517356	
0.66153846	0.15002383	0.12355385	-0.02647	-2.6469981	
0.59230769	0.05835957	-0.0125192	-0.0708788	-7.0878798	
1	1	0.8086	-0.1914	-19.14	**20% w/v**
0.91153846	0.43323498	0.65585346	0.22261849	22.2618486	**exp.3**
0.68461538	0.19995019	0.26402538	0.0640752	6.40751951	
0.59615385	0.16662371	0.11127885	-0.0553449	-5.5344868	
0.56153846	0.09163942	0.05150846	-0.040131	-4.0130954	
1	1	0.9619	-0.0381	-3.81	**25% w/v**
0.88076923	0.68342634	0.72250846	0.03908212	3.90821184	**exp.3**
0.67692308	0.28337955	0.31322615	0.0298466	2.98466045	
0.56153846	0.09169064	0.08155692	-0.0101337	-1.0133718	
0.52307692	0.02501603	0.00433385	-0.0206822	-2.0682185	
1	1	0.8867	-0.1133	-11.33	**30% w/v**
0.89230769	0.58330866	0.69248769	0.10917903	10.9179028	**exp.3**
0.68461538	0.24165162	0.31793538	0.07628376	7.62837601	
0.55	0.08332274	0.07517	-0.0081527	-0.8152744	
0.49615385	0.04165704	-0.0219362	-0.0635932	-6.3593194	
1	1	0.8746	-0.1254	-12.54	**35% w/v**
0.91153846	0.55826114	0.72160577	0.16334463	16.3344631	**exp.3**
0.66923077	0.33329672	0.30253462	-0.0307621	-3.0762105	
0.56923077	0.11665842	0.12958462	0.0129262	1.29261995	
0.51153846	0.05000062	0.02980577	-0.0201948	-2.019485	
1	1	0.9517	-0.0483	-4.83	**15% w/v**
0.82692308	0.44812682	0.53345962	0.0853328	8.53327963	**exp.4**
0.64615385	0.0962079	0.09663077	0.00042287	0.0422869	
0.63846154	0.06421483	0.07804231	0.01382748	1.38274775	
0.61153846	0.06421483	0.01298269	-0.0512321	-5.1232138	
1	1	0.9285	-0.0715	-7.15	**20% w/v**
0.88461538	0.4961159	0.64996154	0.15384563	15.3845634	**exp.4**
0.76153846	0.45612526	0.35285385	-0.1032714	-10.327141	
0.73846154	0.36814501	0.29714615	-0.0709989	-7.0998857	
0.72307692	0.16819127	0.26000769	0.09181642	9.18164241	
1	1	1.0314	0.0314	3.14	**25% w/v**
0.81153846	0.67193885	0.61787769	-0.0540612	-5.406116	**exp.4**
0.60384615	0.16784526	0.16215923	-0.005686	-0.5686027	
0.57307692	0.09583195	0.09464538	-0.0011866	-0.1186562	

0.56153846	0.03982155	0.06932769	0.02950614	2.95061449	
1	1	1.0236	0.0236	2.36	**30% w/v**
0.74615385	0.52794489	0.47288077	-0.0550641	-5.5064118	**exp.4**
0.56923077	0.07989255	0.08904615	0.00915361	0.91536062	
0.55384615	0.03988804	0.05566923	0.01578119	1.57811892	
0.53846154	0.01588527	0.02229231	0.00640704	0.64070391	
1	1	1.022	0.022	2.2	**35% w/v**
0.78076923	0.61597415	0.60907885	-0.0068953	-0.6895307	**exp.4**
0.58846154	0.391959	0.24686731	-0.1450917	-14.509169	
0.55	0.14394236	0.174425	0.03048264	3.04826352	
0.52307692	0.02393434	0.12371538	0.09978104	9.97810412	
1	1	0.9535	-0.0465	-4.65	**15% w/v**
0.87692308	0.51742097	0.59474309	0.07732212	7.73221159	**exp12**
0.71923077	0.13154027	0.13508577	0.0035455	0.35455015	
0.69230769	0.09645973	0.05660769	-0.039852	-3.9852044	
0.68076923	0.01752964	0.02297423	0.00544459	0.54445885	
1	1	1.0152	0.0152	1.52	**20% w/v**
0.84230769	0.62290821	0.58968307	-0.0332251	-3.3225139	**exp.12**
0.69230769	0.15792018	0.18492307	0.02700289	2.70028907	
0.65384615	0.09650684	0.08113845	-0.0153684	-1.5368389	
0.64230769	0.04386659	0.05000307	0.00613648	0.61364807	
1	1	0.9725	-0.0275	-2.75	**25% w/v**
0.78461538	0.38593935	0.44398922	0.05804987	5.80498694	**exp.12**
0.65	0.09648472	0.11367	0.01718528	1.718528	
0.62307692	0.06139945	0.04760615	-0.0137933	-1.3793304	
0.60769231	0.04385682	0.00985539	-0.0340014	-3.400143	
1	1	1.0036	0.0036	0.36	**30% w/v**
0.81538462	0.56149449	0.55057232	-0.0109222	-1.0922171	**exp.12**
0.65	0.13160015	0.144735	0.01313485	1.313485	
0.61538462	0.04386659	0.05979232	0.01592573	1.5925729	
0.6	0.04386659	0.02204	-0.0218266	-2.182659	
1	1	0.944	-0.056	-5.6	**35% w/v**
0.90769231	0.6315785	0.73195077	0.10037227	10.0372275	**exp.12**
0.72692308	0.39473648	0.3166877	-0.0780488	-7.8048781	
0.63461538	0.06140358	0.10463845	0.04323487	4.32348709	
0.61153846	0.06140358	0.05162615	-0.0097774	-0.977743	
1	1	0.8649	-0.1351	-13.51	**15% w/v**
0.93461538	0.50470364	0.67336883	0.16866519	16.8665193	**exp.11**
0.79230769	0.21500162	0.25650692	0.0415053	4.15052963	
0.74615385	0.1682753	0.12130847	-0.0469668	-4.6966827	
0.72692308	0.09351336	0.06497578	-0.0285376	-2.8537582	
1	1	0.8638	-0.1362	-13.62	**20% w/v**
0.92307692	0.49546023	0.66501538	0.16955515	16.9555147	**exp.11**
0.75153846	0.19647359	0.22172569	0.0252521	2.52520983	
0.71538462	0.16884438	0.12829694	-0.0405474	-4.0547449	
0.68846154	0.10304068	0.05872231	-0.0443184	-4.4318368	
1	1	0.9483	-0.0517	-5.17	**25% w/v**
0.90769231	0.63547716	0.69448154	0.05900438	5.90043848	**exp.11**
0.73846154	0.18683431	0.2291477	0.04231339	4.23133865	

0.68461538	0.08401992	0.08108691	-0.002933	-0.293301	
0.66538462	0.07467347	0.02820809	-0.0464654	-4.646538	
1	1	0.9838	-0.0162	-1.62	**30% w/v**
0.86923077	0.62619109	0.64859923	0.02240814	2.24081427	**exp.11**
0.69615385	0.22434656	0.20495116	-0.0193954	-1.9395396	
0.66538462	0.07482308	0.1260804	0.05125732	5.12573164	
0.62692308	0.06547814	0.02749193	-0.0379862	-3.7986209	
1	1	0.9825	-0.0175	-1.75	**35% w/v**
0.86923077	0.62610482	0.64260462	0.0164998	1.64997974	**exp.11**
0.69615385	0.17743041	0.19274309	0.01531268	1.53126769	
0.66538462	0.07460916	0.1127677	0.03815854	3.81585443	
0.62692308	0.06526171	0.01279847	-0.0524632	-5.246324	
1	1	0.9855	-0.0145	-1.45	**15% w/v**
0.68846154	0.16549591	0.23344616	0.06795025	6.79502476	**exp.5**
0.61923077	0.07889632	0.06632308	-0.0125732	-1.2573241	
0.6	0.04740587	0.0199	-0.0275059	-2.750587	
0.59615385	0.02378786	0.01061539	-0.0131725	-1.3172466	
1	1	1.0252	0.0252	2.52	**20% w/v**
0.81923077	0.63773337	0.58582231	-0.0519111	-5.191106	**exp.5**
0.7	0.3148434	0.29602	-0.0188234	-1.88234	
0.67692308	0.27546622	0.23992924	-0.035537	-3.5536982	
0.67307692	0.14946059	0.23058076	0.08112017	8.11201718	
1	1	1.0232	0.0232	2.32	**25% w/v**
0.70384615	0.46458494	0.38999345	-0.0745915	-7.4591487	**exp.5**
0.57692308	0.07877077	0.11861924	0.03984847	3.98484673	
0.55384615	0.06302333	0.06927845	0.00625512	0.62551233	
0.54615385	0.04727589	0.05283155	0.00555566	0.55556567	
1	1	1.0223	0.0223	2.23	**30% w/v**
0.66923077	0.42512381	0.34634	-0.0787838	-7.8783808	**exp.5**
0.54615385	0.05510812	0.09482001	0.03971189	3.97118879	
0.52307692	0.03936255	0.04765999	0.00829744	0.82974437	
0.51538462	0.02361733	0.03194001	0.00832268	0.83226794	
1	1	0.9993	-0.0007	-0.07	**35% w/v**
0.71153846	0.41730819	0.41066538	-0.0066428	-0.6642809	**exp.5**
0.55384615	0.04722018	0.08887845	0.04165827	4.16582737	
0.53076923	0.04722018	0.04178769	-0.0054325	-0.5432489	
0.51153846	0.03147164	0.00254538	-0.0289263	-2.8926259	
1	1	0.9782	-0.0218	-2.18	**15% w/v**
0.75	0.23264406	0.28155	0.04890594	4.89059436	**exp.6**
0.68846154	0.04661786	0.11006692	0.06344906	6.34490587	
0.65384615	0.04661786	0.01360769	-0.0330102	-3.3010172	
0.64230769	0.03886702	-0.0185454	-0.0574124	-5.7412409	
1	1	0.9591	-0.0409	-4.09	**20% w/v**
0.78076923	0.31779093	0.41376346	0.09597253	9.59725295	**exp.6**
0.66153846	0.09297229	0.11717692	0.02420463	2.42046309	
0.63846154	0.09297229	0.05977308	-0.0331992	-3.3199215	
0.62692308	0.07746751	0.03107115	-0.0463964	-4.6396352	
1	1	0.9899	-0.0101	-1.01	**25% w/v**
0.76153846	0.41076018	0.42233769	0.01157751	1.1577515	**exp.6**

0.63846154	0.09288069	0.12940231	0.03652161	3.65216144	
0.60384615	0.04636195	0.04701423	0.00065228	0.06522799	
0.58076923	0.0308557	-0.0079112	-0.0387669	-3.8766857	
1	1	1.0193	0.0193	1.93	**30% w/v**
0.70769231	0.44192277	0.35704769	-0.0848751	-8.4875075	**exp.6**
0.61923077	0.09312458	0.15662923	0.06350465	6.35046505	
0.58076923	0.05436904	0.06949077	0.01512173	1.51217296	
0.56153846	0.03886702	0.02592154	-0.0129455	-1.2945486	
1	1	1.0295	0.0295	2.95	**35% w/v**
0.74230769	0.5348909	0.46025769	-0.0746332	-7.4633212	**exp.6**
0.61923077	0.1860593	0.18838077	0.00232147	0.23214716	
0.58461538	0.10854116	0.11191538	0.00337422	0.33742217	
0.56923077	0.0387749	0.07793077	0.03915587	3.915587	
1	1	0.9455	-0.0545	-5.45	**15% w/v**
0.88461538	0.52593428	0.61319231	0.08725803	8.72580284	**exp.9**
0.70769231	0.11220388	0.10365385	-0.00855	-0.8550037	
0.68846154	0.07772666	0.04826923	-0.0294574	-2.9457431	
0.68846154	0.04324869	0.04826923	0.00502054	0.50205377	
1	1	0.9888	-0.0112	-1.12	**20% w/v**
0.85	0.58619272	0.604785	0.01859228	1.85922829	**exp.9**
0.66923077	0.1379014	0.14199769	0.00409629	0.40962917	
0.63846154	0.10341737	0.06322538	-0.040192	-4.0191982	
0.63846154	0.0344493	0.06322538	0.02877608	2.87760849	
1	1	1.0237	0.0237	2.37	**25% w/v**
0.79615385	0.57752443	0.52164731	-0.0558771	-5.5877121	**exp.9**
0.65384615	0.14642671	0.17115769	0.02473098	2.47309778	
0.61923077	0.07745092	0.08590346	0.00845254	0.84525427	
0.61538462	0.07745092	0.07643077	-0.0010201	-0.102015	
1	1	1.0666	0.0666	6.66	**30% w/v**
0.84615385	0.79298544	0.67813846	-0.114847	-11.484698	**exp.9**
0.64230769	0.1292935	0.16342692	0.03413342	3.41334205	
0.61153846	0.0689578	0.08573462	0.01677681	1.67768111	
0.60384615	0.0689578	0.06631154	-0.0026463	-0.2646266	
1	1	0.9644	-0.0356	-3.56	**35% w/v**
0.92692308	0.75863156	0.80656115	0.04792959	4.79295917	**exp.9**
0.63846154	0.21555282	0.18351308	-0.0320397	-3.203974	
0.60769231	0.11210948	0.11705462	0.00494514	0.49451404	
0.58846154	0.06038764	0.07551808	0.01513043	1.51304329	
1	1	0.8907	-0.1093	-10.93	**15% w/v**
0.95769231	0.65055677	0.75248923	0.10193246	10.193246	**exp.10**
0.80769231	0.17492515	0.26246923	0.08754408	8.75440836	
0.75	0.09727082	0.074	-0.0232708	-2.3270816	
0.73076923	0.06815055	0.01117692	-0.0569736	-5.6973624	
1	1	0.9884	-0.0116	-1.16	**20% w/v**
0.93846154	0.74757098	0.82780308	0.0802321	8.0232099	**exp.10**
0.75384615	0.62135647	0.34601231	-0.2753442	-27.534416	
0.70384615	0.11649842	0.21552731	0.09902888	9.9028885	
0.68846154	0.06795457	0.17537808	0.1074235	10.7423503	
1	1	0.9847	-0.0153	-1.53	**25% w/v**

0.92692308	0.73782887	0.78123923	0.04341036	4.34103565	**exp.10**
0.74230769	0.35913781	0.26723308	-0.0919047	-9.1904732	
0.68461538	0.06783645	0.10660615	0.03876971	3.87697076	
0.66923077	0.03870671	0.06377231	0.02506559	2.50655939	
1	1	1.0584	0.0584	5.84	**30% w/v**
0.91153846	0.89301514	0.81156577	-0.0814494	-8.144937	**exp.10**
0.71538462	0.2523688	0.26423769	0.01186889	1.1868895	
0.66538462	0.11647393	0.12472269	0.00824876	0.82487646	
0.64615385	0.06793987	0.07106308	0.00312321	0.31232115	
1	1	1.016	0.016	1.6	**35% w/v**
0.91923077	0.82525703	0.81056346	-0.0146936	-1.4693568	**exp.10**
0.69230769	0.27190483	0.23338462	-0.0385202	-3.8520215	
0.65	0.08745422	0.125775	0.03832078	3.83207823	
0.63076923	0.07774621	0.07686154	-0.0008847	-0.0884676	
1	1	1.0267	0.0267	2.67	**15% w/v**
0.70769231	0.4180142	0.31025385	-0.1077604	-10.776036	**exp.8**
0.61923077	0.0573473	0.09343462	0.03608731	3.60873142	
0.60769231	0.03275639	0.06515385	0.03239745	3.23974541	
0.59615385	0.02455966	0.03687308	0.01231342	1.23134178	
1	1	0.991	-0.009	-0.9	**20% w/v**
0.72307692	0.35232339	0.37196615	0.01964277	1.96427655	**exp.8**
0.59615385	0.05718007	0.08824231	0.03106223	3.10622331	
0.56923077	0.05718007	0.02805846	-0.0291216	-2.9121613	
0.56923077	0.04078316	0.02805846	-0.0127247	-1.2724703	
1	1	1.0106	0.0106	1.06	**25% w/v**
0.66538462	0.37711802	0.33447615	-0.0426419	-4.2641869	**exp.8**
0.58076923	0.15583085	0.16350231	0.00767146	0.76714598	
0.55	0.0820686	0.10133	0.0192614	1.92614018	
0.53076923	0.05748147	0.06247231	0.00499084	0.4990842	
1	1	1.048	0.048	4.8	**30% w/v**
0.71923077	0.59015092	0.45481885	-0.1353321	-13.533208	**exp.8**
0.57692308	0.09833239	0.15416538	0.055833	5.58329983	
0.54230769	0.04915057	0.08103346	0.03188289	3.18828934	
0.52692308	0.04915057	0.04853038	-0.0006202	-0.0620184	
1	1	1.0412	0.0412	4.12	**35% w/v**
0.75	0.59830715	0.5082	-0.0901072	-9.0107154	**exp.8**
0.55384615	0.07364749	0.09	0.01635251	1.63525114	
0.53461538	0.02446058	0.049	0.02453942	2.45394216	
0.52692308	0.02446058	0.0326	0.00813942	0.81394216	
1	1	0.9598	-0.0402	-4.02	**15% w/v**
0.79230769	0.33569703	0.43365308	0.09795605	9.79560497	**exp.7**
0.65769231	0.1239962	0.09263192	-0.0313643	-3.1364282	
0.64615385	0.06559646	0.06340154	-0.0021949	-0.2194919	
0.63461538	0.05829602	0.03417115	-0.0241249	-2.4124861	
1	1	0.992	-0.008	-0.8	**20% w/v**
0.75769231	0.40869782	0.40847462	-0.0002232	-0.0223202	**exp.7**
0.65	0.09479649	0.14913	0.05433351	5.43335104	
0.60769231	0.0509962	0.04724462	-0.0037516	-0.375159	
0.58846154	0.04369592	0.00093308	-0.0427628	-4.2762843	

1	1	0.9997	-0.0003	-0.03	**25% w/v**
0.73846154	0.40139772	0.39612154	-0.0052762	-0.5276184	**exp.8**
0.62307692	0.11669639	0.12983692	0.01314053	1.31405284	
0.60384615	0.07289639	0.08545615	0.01255976	1.25597592	
0.57692308	0.04369602	0.02332308	-0.0203729	-2.0372938	
1	1	1.0476	0.0476	4.76	**30% w/v**
0.71538462	0.56902563	0.43152154	-0.1375041	-13.750409	**exp.7**
0.58846154	0.18187911	0.15678385	-0.0250953	-2.5095268	
0.58076923	0.07961424	0.14013308	0.06051884	6.05188364	
0.55769231	0.03578608	0.09018077	0.05439469	5.43946933	
1	1	1.0604	0.0604	6.04	**35% w/v**
0.71923077	0.62039848	0.45843077	-0.1619677	-16.196771	**exp.7**
0.58461538	0.18969667	0.16981538	-0.0198813	-1.9881281	
0.56538462	0.05829629	0.12858462	0.07028833	7.02883291	
0.54615385	0.03639604	0.08735385	0.0509578	5.09578039	

Note:

(i). Experiments no 1, 2 , 3, and 4 were conducted with conditions:
air flow rate of 1.0 litre/minute and stirrer speed of 125 rpm.

(ii). Experiments no 5, 6 , 11, and 12 were conducted with conditions:
air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

(iii). Experiments no 7, 8 , 9, and 10 were conducted with conditions:
air flow rate of 1.5 litre/minute and stirrer speed of 175 rpm

APPENDIX G

PREDICTED AND EXPERIMENTAL PYRITIC SULPHUR REMOVAL (BASED ON pH EQUATION)

Day	C_o (mM/l)	C_t calculated (mM/l)	C_t exp. (mM/l)	ΣPy cal-removed (ΔC_tcal) (mM/l)	ΣPy exp removed (ΔC_texp) (mM/l)	Experiment conditions	Abs. ΔC_t removed (mM/l)
6	13.39	10.20	8.08	3.19	5.31	15% w/v	2.12
12		6.10	4.96	7.29	8.43	30°C	1.14
18		4.29	4.85	9.10	8.54	1.0 l/min	0.55
24		3.82	4.39	9.57	9.00	125 rpm	0.57
6	17.85	14.05	11.39	3.80	6.46	20% w/v	2.67
12		7.71	8.46	10.14	9.39	30°C	0.76
18		5.37	4.77	12.48	13.08	1.0 l/min	0.60
24		4.21	4.62	13.64	13.23	125 rpm	0.41
6	22.31	16.90	12.12	5.41	10.19	25% w/v	4.78
12		8.19	9.23	14.12	13.08	30°C	1.04
18		4.80	4.04	17.51	18.27	1.0 l/min	0.76
24		3.62	4.04	18.69	18.27	125 rpm	0.42
6	26.78	24.83	23.31	1.95	3.47	30% w/v	1.52
12		12.94	15.23	13.84	11.55	30°C	2.30
18		6.55	5.54	20.23	21.24	1.0 l/min	1.01
24		4.79	4.39	21.99	22.39	125 rpm	0.40
6	31.24	29.73	27.20	1.51	4.04	35% w/v	2.53
12		18.94	25.04	12.30	6.20	30°C	6.10
18		14.56	14.81	16.68	16.43	1.0 l/min	0.25
24		13.04	8.89	18.20	22.35	125 rpm	4.15
6	13.04	9.20	9.00	3.84	4.04	15% w/v	0.20
12		3.13	2.31	9.91	10.73	35°C	0.83
18		1.21	0.69	11.83	12.35	1.0 l/min	0.52
24		-0.42	0.69	13.46	12.35	125 rpm	1.11
6	17.39	9.73	8.93	7.66	8.46	20% w/v	0.81
12		4.21	3.85	13.18	13.54	35°C	0.37
18		2.01	1.23	15.38	16.16	1.0 l/min	0.78
24		-0.20	1.08	17.59	16.31	125 rpm	1.28
6	21.74	8.98	9.43	12.76	12.31	25% w/v	0.45
12		4.37	2.50	17.37	19.24	35°C	1.87
18		1.30	1.35	20.44	20.39	1.0 l/min	0.05
24		-1.12	0.00	22.86	21.74	125 rpm	1.12
6	26.08	11.47	10.38	14.61	15.70	30% w/v	1.09
12		4.22	3.46	21.86	22.62	35°C	0.76
18		2.15	2.07	23.93	24.01	1.0 l/min	0.07
24		0.07	1.38	26.01	24.70	125 rpm	1.31
6	30.43	17.44	15.62	12.99	14.81	35% w/v	1.82
12		11.80	13.73	18.63	16.70	35°C	1.93
18		8.05	8.08	22.38	22.35	1.0 l/min	0.03

24		5.37	4.85	25.06	25.58	125 rpm	0.52
6	13.85	8.62	5.19	5.23	8.66	15% w/v	3.43
12		3.39	2.54	10.46	11.31	40°C	0.85
18		1.71	2.08	12.14	11.77	1.0 l/min	0.37
24		-0.17	0.81	14.02	13.04	125 rpm	0.98
6	18.47	12.11	8.00	6.36	10.47	20% w/v	4.11
12		4.88	3.69	13.59	14.78	40°C	1.18
18		2.06	3.08	16.41	15.39	1.0 l/min	1.02
24		0.95	1.69	17.52	16.78	125 rpm	0.74
6	23.08	16.68	15.77	6.40	7.31	25% w/v	0.90
12		7.23	6.54	15.85	16.54	40°C	0.69
18		1.88	2.12	21.20	20.96	1.0 l/min	0.23
24		0.10	0.58	22.98	22.50	125 rpm	0.48
6	27.70	19.18	16.16	8.52	11.54	30% w/v	3.02
12		8.81	6.69	18.89	21.01	40°C	2.11
18		2.08	2.31	25.62	25.39	1.0 l/min	0.23
24		-0.61	1.15	28.31	26.55	125 rpm	1.76
6	32.32	23.32	18.04	9.00	14.28	35% w/v	5.28
12		9.78	10.77	22.54	21.55	40°C	0.99
18		4.19	3.77	28.13	28.55	1.0 l/min	0.42
24		0.96	1.62	31.36	30.70	125 rpm	0.65
6	14.43	7.70	6.47	6.73	7.96	15% w/v	1.23
12		1.39	1.39	13.04	13.04	45°C	0.01
18		1.13	0.93	13.30	13.50	1.0 l/min	0.20
24		0.19	0.93	14.24	13.50	125 rpm	0.74
6	19.24	12.51	9.55	6.73	9.69	20% w/v	2.96
12		6.79	8.78	12.45	10.46	45°C	1.99
18		5.72	7.08	13.52	12.16	1.0 l/min	1.37
24		5.00	3.24	14.24	16.00	125 rpm	1.77
6	24.04	14.85	16.15	9.19	7.89	25% w/v	1.30
12		3.90	4.03	20.14	20.01	45°C	0.14
18		2.28	2.30	21.76	21.74	1.0 l/min	0.03
24		1.67	0.96	22.37	23.08	125 rpm	0.71
6	28.85	13.64	15.23	15.21	13.62	30% w/v	1.59
12		2.57	2.30	26.28	26.55	45°C	0.26
18		1.61	1.15	27.24	27.70	1.0 l/min	0.46
24		0.64	0.46	28.21	28.39	125 rpm	0.18
6	33.66	20.50	20.73	13.16	12.93	35% w/v	0.23
12		8.31	13.19	25.35	20.47	45°C	4.88
18		5.87	4.85	27.79	28.81	1.0 l/min	1.03
24		4.16	0.81	29.50	32.85	125 rpm	3.36
6	13.16	7.83	6.81	5.33	6.35	15% w/v	1.02
12		1.78	1.73	11.38	11.43	35°C	0.05
18		0.74	1.27	12.42	11.89	1.5 l/min	0.52
24		0.30	0.23	12.86	12.93	125 rpm	0.07
6	17.54	10.34	10.93	7.20	6.61	20% w/v	0.58
12		3.24	2.77	14.30	14.77	35°C	0.47
18		1.42	1.69	16.12	15.85	1.5 l/min	0.27

24		0.88	0.77	16.66	16.77	125 rpm	0.11
6	21.93	9.74	8.46	12.19	13.47	25% w/v	1.27
12		2.49	2.12	19.44	19.81	35°C	0.38
18		1.04	1.35	20.89	20.58	1.5 l/min	0.30
24		0.22	0.96	21.71	20.97	125 rpm	0.75
6	26.31	14.49	14.77	11.82	11.54	30% w/v	0.29
12		3.81	3.46	22.50	22.85	35°C	0.35
18		1.57	1.15	24.74	25.16	1.5 l/min	0.42
24		0.58	1.15	25.73	25.16	125 rpm	0.57
6	30.70	22.47	19.39	8.23	11.31	35% w/v	3.08
12		9.72	12.12	20.98	18.58	35°C	2.40
18		3.21	1.89	27.49	28.81	1.5 l/min	1.33
24		1.58	1.89	29.12	28.81	125 rpm	0.30
6	12.35	8.32	6.23	4.03	6.12	15% w/v	2.08
12		3.17	2.66	9.18	9.69	30°C	0.51
18		1.50	2.08	10.85	10.27	1.5 l/min	0.58
24		0.80	1.15	11.55	11.20	125 rpm	0.35
6	16.47	10.95	8.16	5.52	8.31	20% w/v	2.79
12		3.65	3.24	12.82	13.23	30°C	0.42
18		2.11	2.78	14.36	13.69	1.5 l/min	0.67
24		0.97	1.70	15.50	14.77	125 rpm	0.73
6	20.58	14.29	13.08	6.29	7.50	25% w/v	1.21
12		4.72	3.85	15.86	16.73	30°C	0.87
18		1.67	1.73	18.91	18.85	1.5 l/min	0.06
24		0.58	1.54	20.00	19.04	125 rpm	0.96
6	24.70	16.02	15.47	8.68	9.23	30% w/v	0.55
12		5.06	5.54	19.64	19.16	30°C	0.48
18		3.11	1.85	21.59	22.85	1.5 l/min	1.27
24		0.68	1.62	24.02	23.08	125 rpm	0.94
6	28.81	18.51	18.04	10.30	10.77	35% w/v	0.48
12		5.55	5.11	23.26	23.70	30°C	0.44
18		3.25	2.15	25.56	26.66	1.5 l/min	1.10
24		0.37	1.88	28.44	26.93	125 rpm	1.51
6	14.66	3.42	2.43	11.24	12.23	15% w/v	1.00
12		0.97	1.16	13.69	13.50	45°C	0.18
18		0.29	0.69	14.37	13.97	1.5 l/min	0.40
24		0.16	0.35	14.50	14.31	125 rpm	0.19
6	19.54	11.45	12.46	8.09	7.08	20% w/v	1.01
12		5.78	6.15	13.76	13.39	45°C	0.37
18		4.69	5.38	14.85	14.16	1.5 l/min	0.69
24		4.51	2.92	15.03	16.62	125 rpm	1.59
6	24.43	9.53	11.35	14.90	13.08	25% w/v	1.82
12		2.90	1.92	21.53	22.51	45°C	0.97
18		1.69	1.54	22.74	22.89	1.5 l/min	0.15
24		1.29	1.15	23.14	23.28	125 rpm	0.14
6	29.32	10.15	12.46	19.17	16.86	30% w/v	2.31
12		2.78	1.62	26.54	27.70	45°C	1.16
18		1.40	1.15	27.92	28.17	1.5 l/min	0.24

24		0.94	0.69	28.38	28.63	125 rpm	0.24
6	34.20	14.04	14.27	20.16	19.93	35% w/v	0.23
12		3.04	1.61	31.16	32.59	45°C	1.42
18		1.43	1.61	32.77	32.59	1.5 l/min	0.19
24		0.09	1.08	34.11	33.12	125 rpm	0.99
6	14.89	4.19	3.46	10.70	11.43	15% w/v	0.73
12		1.64	0.69	13.25	14.20	40°C	0.94
18		0.20	0.69	14.69	14.20	1.5 l/min	0.49
24		-0.28	0.58	15.17	14.31	125 rpm	0.85
6	19.85	8.21	6.31	11.64	13.54	20% w/v	1.91
12		2.33	1.85	17.52	18.00	40°C	0.48
18		1.19	1.85	18.66	18.00	1.5 l/min	0.66
24		0.62	1.54	19.23	18.31	125 rpm	0.92
6	24.81	10.48	10.19	14.33	14.62	25% w/v	0.29
12		3.21	2.30	21.60	22.51	40°C	0.91
18		1.17	1.15	23.64	23.66	1.5 l/min	0.02
24		-0.20	0.77	25.01	24.04	125 rpm	0.96
6	29.78	10.63	13.16	19.15	16.62	30% w/v	2.53
12		4.66	2.77	25.12	27.01	40°C	1.89
18		2.07	1.62	27.71	28.16	1.5 l/min	0.45
24		0.77	1.16	29.01	28.62	125 rpm	0.39
6	34.74	15.99	18.58	18.75	16.16	35% w/v	2.59
12		6.54	6.46	28.20	28.28	40°C	0.08
18		3.89	3.77	30.85	30.97	1.5 l/min	0.12
24		2.71	1.35	32.03	33.39	125 rpm	1.36
6	13.39	8.21	7.04	5.18	6.35	15% w/v	1.17
12		1.39	1.50	12.00	11.89	35°C	0.11
18		0.65	1.04	12.74	12.35	1.5 l/min	0.39
24		0.65	0.58	12.74	12.81	175 rpm	0.07
6	17.85	10.80	10.46	7.05	7.39	20% w/v	0.33
12		2.53	2.46	15.32	15.39	35°C	0.07
18		1.13	1.85	16.72	16.00	1.5 l/min	0.72
24		1.13	0.61	16.72	17.24	175 rpm	0.51
6	22.31	11.64	12.88	10.67	9.43	25% w/v	1.25
12		3.82	3.27	18.49	19.04	35°C	0.55
18		1.92	1.73	20.39	20.58	1.5 l/min	0.19
24		1.71	1.73	20.60	20.58	175 rpm	0.02
6	26.78	18.16	21.24	8.62	5.54	30% w/v	3.08
12		4.38	3.46	22.40	23.32	35°C	0.91
18		2.30	1.85	24.48	24.93	1.5 l/min	0.45
24		1.78	1.85	25.00	24.93	175 rpm	0.07
6	31.24	25.20	23.70	6.04	7.54	35% w/v	1.50
12		5.73	6.73	25.51	24.51	35°C	1.00
18		3.66	3.50	27.58	27.74	1.5 l/min	0.15
24		2.36	1.89	28.88	29.35	175 rpm	0.47
6	11.89	8.95	7.74	2.94	4.15	15% w/v	1.21
12		3.12	2.08	8.77	9.81	30°C	1.04
18		0.88	1.16	11.01	10.73	1.5 l/min	0.28

24		0.13	0.81	11.76	11.08	175 rpm	0.68
6	15.85	13.12	11.85	2.73	4.00	20% w/v	1.27
12		5.48	9.85	10.37	6.00	30°C	4.36
18		3.42	1.85	12.43	14.00	1.5 l/min	1.57
24		2.78	1.08	13.07	14.77	175 rpm	1.70
6	19.81	15.48	14.62	4.33	5.19	25% w/v	0.86
12		5.29	7.11	14.52	12.70	30°C	1.82
18		2.11	1.34	17.70	18.47	1.5 l/min	0.77
24		1.26	0.77	18.55	19.04	175 rpm	0.50
6	23.78	19.30	21.24	4.48	2.54	30% w/v	1.94
12		6.28	6.00	17.50	17.78	30°C	0.28
18		2.97	2.77	20.81	21.01	1.5 l/min	0.20
24		1.69	1.62	22.09	22.16	175 rpm	0.07
6	27.74	22.49	22.89	5.25	4.85	35% w/v	0.41
12		6.47	7.54	21.27	20.20	30°C	1.07
18		3.49	2.43	24.25	25.31	1.5 l/min	1.06
24		2.13	2.16	25.61	25.58	175 rpm	0.02
6	14.08	4.37	5.89	9.71	8.19	15% w/v	1.52
12		1.32	0.81	12.76	13.27	45°C	0.51
18		0.92	0.46	13.16	13.62	1.5 l/min	0.46
24		0.52	0.35	13.56	13.73	175 rpm	0.17
6	18.77	6.98	6.61	11.79	12.16	20% w/v	0.37
12		1.66	1.07	17.11	17.70	45°C	0.58
18		0.53	1.07	18.24	17.70	1.5 l/min	0.55
24		0.53	0.77	18.24	18.00	175 rpm	0.24
6	23.47	7.85	8.85	15.62	14.62	25% w/v	1.00
12		3.84	3.66	19.63	19.81	45°C	0.18
18		2.38	1.93	21.09	21.54	1.5 l/min	0.45
24		1.47	1.35	22.00	22.12	175 rpm	0.12
6	28.16	12.81	16.62	15.35	11.54	30% w/v	3.81
12		4.34	2.77	23.82	25.39	45°C	1.57
18		2.28	1.38	25.88	26.78	1.5 l/min	0.90
24		1.37	1.38	26.79	26.78	175 rpm	0.02
6	32.85	16.69	19.65	16.16	13.20	35% w/v	2.96
12		2.96	2.42	29.89	30.43	45°C	0.54
18		1.61	0.80	31.24	32.05	1.5 l/min	0.81
24		1.07	0.80	31.78	32.05	175 rpm	0.27
6	15.81	6.86	5.31	8.95	10.50	15% w/v	1.55
12		1.46	1.96	14.35	13.85	40°C	0.50
18		1.00	1.04	14.81	14.77	1.5 l/min	0.03
24		0.54	0.92	15.27	14.89	175 rpm	0.38
6	21.08	8.61	8.62	12.47	12.46	20% w/v	0.00
12		3.14	2.00	17.94	19.08	40°C	1.15
18		1.00	1.07	20.08	20.01	1.5 l/min	0.08
24		0.02	0.92	21.06	20.16	175 rpm	0.90
6	26.35	10.44	10.58	15.91	15.77	25% w/v	0.14
12		3.42	3.07	22.93	23.28	40°C	0.35
18		2.25	1.92	24.10	24.43	1.5 l/min	0.33

24		0.61	1.15	25.74	25.20	175 rpm	0.54
6	31.60	13.64	17.98	17.96	13.62	30% w/v	4.35
12		4.95	5.75	26.65	25.85	40°C	0.79
18		4.43	2.52	27.17	29.08	1.5 l/min	1.91
24		2.85	1.13	28.75	30.47	175 rpm	1.72
6	36.89	16.91	22.89	19.98	14.00	35% w/v	5.97
12		6.26	7.00	30.63	29.89	40°C	0.73
18		4.74	2.15	32.15	34.74	1.5 l/min	2.59
24		3.22	1.34	33.67	35.55	175 rpm	1.88

APPENDIX H

CALCULATION OF PYRITIC SULPHUR REMOVAL BASED ON pH EQUATION FOR EXPERIMENTS AT 1.5 LITRE/MINUTE, 175 RPM, 25% W/V, AND TEMPERATURE RANGE: (30-45)^{O}C

pH_t	pH_o	C_o(M/l)	∂	ß	C_t(M/l)	Pyrmv(M/l)	Pyrmv(mg/l)
2.6	2.6	0.01981	-1.7995	2.7842	0.01950691	0.00030309	36.3620672
2.6	2.6	0.01981	-1.7995	2.7842	0.01950691	0.00030309	36.3620672
2.57	2.6	0.01981	-1.7995	2.7842	0.0188705	0.0009395	112.711439
2.56	2.6	0.01981	-1.7995	2.7842	0.01865837	0.00115163	138.16123
2.45	2.6	0.01981	-1.7995	2.7842	0.01632489	0.00348511	418.108928
2.41	2.6	0.01981	-1.7995	2.7842	0.01547635	0.00433365	519.908091
2.29	2.6	0.01981	-1.7995	2.7842	0.01293073	0.00687927	825.30558
2.19	2.6	0.01981	-1.7995	2.7842	0.01080939	0.00900061	1079.80349
2.1	2.6	0.01981	-1.7995	2.7842	0.00890018	0.01090982	1308.8516
2.04	2.6	0.01981	-1.7995	2.7842	0.00762737	0.01218263	1461.55035
1.97	2.6	0.01981	-1.7995	2.7842	0.00614243	0.01366757	1639.69888
1.93	2.6	0.01981	-1.7995	2.7842	0.00529389	0.01451611	1741.49805
1.87	2.6	0.01981	-1.7995	2.7842	0.00402108	0.01578892	1894.19679
1.83	2.6	0.01981	-1.7995	2.7842	0.00317254	0.01663746	1995.99595
1.83	2.6	0.01981	-1.7995	2.7842	0.00317254	0.01663746	1995.99595
1.8	2.6	0.01981	-1.7995	2.7842	0.00253614	0.01727386	2072.34533
1.79	2.6	0.01981	-1.7995	2.7842	0.002324	0.017486	2097.79512
1.78	2.6	0.01981	-1.7995	2.7842	0.00211187	0.01769813	2123.24491
1.76	2.6	0.01981	-1.7995	2.7842	0.0016876	0.0181224	2174.14449
1.74	2.6	0.01981	-1.7995	2.7842	0.00126333	0.01854667	2225.04407
1.74	2.6	0.01981	-1.7995	2.7842	0.00126333	0.01854667	2225.04407
1.74	2.6	0.01981	-1.7995	2.7842	0.00126333	0.01854667	2225.04407
1.74	2.6	0.01981	-1.7995	2.7842	0.00126333	0.01854667	2225.04407
1.74	2.6	0.01981	-1.7995	2.7842	0.00126333	0.01854667	2225.04407
2.6	2.6	0.02231	-1.4392	2.4629	0.02283875	-0.0005287	-63.433778
2.55	2.6	0.02231	-1.4392	2.4629	0.02178207	0.00052793	63.3359813
2.51	2.6	0.02231	-1.4392	2.4629	0.02093673	0.00137327	164.751788
2.5	2.6	0.02231	-1.4392	2.4629	0.02072539	0.00158461	190.10574
2.31	2.6	0.02231	-1.4392	2.4629	0.01671001	0.00559999	671.830824
2.07	2.6	0.02231	-1.4392	2.4629	0.01163795	0.01067205	1280.32567
1.99	2.6	0.02231	-1.4392	2.4629	0.00994727	0.01236273	1483.15728
1.87	2.6	0.02231	-1.4392	2.4629	0.00741124	0.01489876	1787.4047
1.78	2.6	0.02231	-1.4392	2.4629	0.00550921	0.01680079	2015.59027
1.74	2.6	0.02231	-1.4392	2.4629	0.00466387	0.01764613	2117.00607
1.72	2.6	0.02231	-1.4392	2.4629	0.0042412	0.0180688	2167.71398
1.7	2.6	0.02231	-1.4392	2.4629	0.00381853	0.01849147	2218.42188
1.67	2.6	0.02231	-1.4392	2.4629	0.00318452	0.01912548	2294.48374
1.65	2.6	0.02231	-1.4392	2.4629	0.00276185	0.01954815	2345.19164
1.65	2.6	0.02231	-1.4392	2.4629	0.00276185	0.01954815	2345.19164
1.62	2.6	0.02231	-1.4392	2.4629	0.00212784	0.02018216	2421.2535
1.61	2.6	0.02231	-1.4392	2.4629	0.00191651	0.02039349	2446.60745

1.61	2.6	0.02231	-1.4392	2.4629	0.00191651	0.02039349	2446.60745
1.61	2.6	0.02231	-1.4392	2.4629	0.00191651	0.02039349	2446.60745
1.61	2.6	0.02231	-1.4392	2.4629	0.00191651	0.02039349	2446.60745
1.6	2.6	0.02231	-1.4392	2.4629	0.00170517	0.02060483	2471.9614
1.6	2.6	0.02231	-1.4392	2.4629	0.00170517	0.02060483	2471.9614
1.6	2.6	0.02231	-1.4392	2.4629	0.00170517	0.02060483	2471.9614
1.6	2.6	0.02231	-1.4392	2.4629	0.00170517	0.02060483	2471.9614
2.6	2.6	0.02635	-1.3081	2.3078	0.0263421	7.905E-06	0.94836285
2.58	2.6	0.02635	-1.3081	2.3078	0.02587432	0.00047568	57.0671266
2.58	2.6	0.02635	-1.3081	2.3078	0.02587432	0.00047568	57.0671266
2.46	2.6	0.02635	-1.3081	2.3078	0.02306768	0.00328232	393.779709
2.17	2.6	0.02635	-1.3081	2.3078	0.01628497	0.01006503	1207.50178
1.92	2.6	0.02635	-1.3081	2.3078	0.0104378	0.0159122	1908.98633
1.81	2.6	0.02635	-1.3081	2.3078	0.00786505	0.01848495	2217.63953
1.75	2.6	0.02635	-1.3081	2.3078	0.00646173	0.01988827	2385.99582
1.68	2.6	0.02635	-1.3081	2.3078	0.00482452	0.02152548	2582.41149
1.68	2.6	0.02635	-1.3081	2.3078	0.00482452	0.02152548	2582.41149
1.65	2.6	0.02635	-1.3081	2.3078	0.00412286	0.02222714	2666.58964
1.62	2.6	0.02635	-1.3081	2.3078	0.0034212	0.0229288	2750.76779
1.61	2.6	0.02635	-1.3081	2.3078	0.00318732	0.02316268	2778.82717
1.57	2.6	0.02635	-1.3081	2.3078	0.00225177	0.02409823	2891.06469
1.57	2.6	0.02635	-1.3081	2.3078	0.00225177	0.02409823	2891.06469
1.57	2.6	0.02635	-1.3081	2.3078	0.00225177	0.02409823	2891.06469
1.57	2.6	0.02635	-1.3081	2.3078	0.00225177	0.02409823	2891.06469
1.57	2.6	0.02635	-1.3081	2.3078	0.00225177	0.02409823	2891.06469
1.57	2.6	0.02635	-1.3081	2.3078	0.00225177	0.02409823	2891.06469
1.53	2.6	0.02635	-1.3081	2.3078	0.00131622	0.02503378	3003.30222
1.5	2.6	0.02635	-1.3081	2.3078	0.00061456	0.02573544	3087.48037
1.5	2.6	0.02635	-1.3081	2.3078	0.00061456	0.02573544	3087.48037
1.5	2.6	0.02635	-1.3081	2.3078	0.00061456	0.02573544	3087.48037
1.5	2.6	0.02635	-1.3081	2.3078	0.00061456	0.02573544	3087.48037
2.6	2.6	0.02347	-1.01	2.0206	0.02371878	-0.0002488	-29.846377
2.57	2.6	0.02347	-1.01	2.0206	0.02317159	0.00029841	35.8004904
2.54	2.6	0.02347	-1.01	2.0206	0.02262439	0.00084561	101.447357
2.32	2.6	0.02347	-1.01	2.0206	0.01861164	0.00485836	582.857715
1.98	2.6	0.02347	-1.01	2.0206	0.01241011	0.01105989	1326.85554
1.73	2.6	0.02347	-1.01	2.0206	0.00785016	0.01561984	1873.91276
1.71	2.6	0.02347	-1.01	2.0206	0.00748536	0.01598464	1917.67734
1.6	2.6	0.02347	-1.01	2.0206	0.00547898	0.01799102	2158.38252
1.56	2.6	0.02347	-1.01	2.0206	0.00474939	0.01872061	2245.91168
1.52	2.6	0.02347	-1.01	2.0206	0.0040198	0.0194502	2333.44083
1.51	2.6	0.02347	-1.01	2.0206	0.0038374	0.0196326	2355.32312
1.51	2.6	0.02347	-1.01	2.0206	0.0038374	0.0196326	2355.32312
1.46	2.6	0.02347	-1.01	2.0206	0.00292541	0.02054459	2464.73457
1.46	2.6	0.02347	-1.01	2.0206	0.00292541	0.02054459	2464.73457
1.46	2.6	0.02347	-1.01	2.0206	0.00292541	0.02054459	2464.73457
1.46	2.6	0.02347	-1.01	2.0206	0.00292541	0.02054459	2464.73457
1.43	2.6	0.02347	-1.01	2.0206	0.00237822	0.02109178	2530.38143
1.43	2.6	0.02347	-1.01	2.0206	0.00237822	0.02109178	2530.38143

1.43	2.6	0.02347	-1.01	2.0206	0.00237822	0.02109178	2530.38143
1.42	2.6	0.02347	-1.01	2.0206	0.00219582	0.02127418	2552.26372
1.39	2.6	0.02347	-1.01	2.0206	0.00164862	0.02182138	2617.91059
1.39	2.6	0.02347	-1.01	2.0206	0.00164862	0.02182138	2617.91059
1.38	2.6	0.02347	-1.01	2.0206	0.00146623	0.02200377	2639.79288
1.38	2.6	0.02347	-1.01	2.0206	0.00146623	0.02200377	2639.79288

APPENDIX I :

ADDED VALUE OF ACID WATER PRODUCED FROM MICROBIAL COAL DESULPHURISATION

1. Recovery of sulphuric acid from acid water.

The sulphuric acid as a product of microorganisms' activity during the pyritic sulphur oxidation can be recovered and is capable of being processed for resale as gypsum. The sulphuric acid is neutralized with calcium compounds (natural limestone, quicklime, or calcium oxide, slaked lime, calcium hydroxide, and carbide residue), ultimately producing gypsum. The reaction for this recovery is:

$$H_2SO_4 + CaCO_3 + H_2O \text{ ----> } CaSO_4.2H_2O_{(s)} + CO_{2(g)}$$

2. Potential agricultural uses of sulphuric acid, include reclamation of sodic soils to increase plant nutrient availability, treatment of alkaline or ammoniated irrigation water; control of certain weeds and pathogens; and improvement of range grass establishment.

Note: If the sulphuric acid will not be recovered, the acid water should be treated before it is recycled. Methods for treating acid water include reverse osmosis, demineralization, ion exchange, active biochemical sludge followed by limestone neutralization, and hydrated lime neutralization. The hydrated lime neutralization is effective and is the only extensively used method of treating acid mine drainage.

APPENDIX J:

EQUATIONS FROM PLOTS ln C_o/C_t VERSUS TIME

Experimental conditions	Equations derived from plots ln C_o/C_t vs time	
15% w/v; 1.0 litre/minute; 125 rpm		
30°C	$y = 0.17732 + 1.9048e^{-3}x$	R^2= 0.852
35°C	$y = -9.2848e^{-2} + 5.8633e^{-3}x$	R^2= 0.930
40°C	$y = 0.16335 + 4.5823e^{-3}x$	R^2= 0.965
45°C	$y = 0.24022 + 5.1624e^{-3}x$	R^2= 0.879
20% w/v; 1.0 litre/minute; 125 rpm		
30°C	$y = 5.8591e^{-2} + 2.4825e^{-3}x$	R^2= 0.956
35°C	$y = 1.2301e^{-2} + 5.2371e^{-3}x$	R^2= 0.964
40°C	$y = 0.17846 + 3.9826e^{-3}x$	R^2= 0.960
45°C	$y = 8.0845e^{-2} + 2.6831e^{-3}x$	R^2= 0.879
25% w/v; 1.0 litre/minute; 125 rpm		
30°C	$y = 7.8846e^{-2} + 3.1366e^{-3}x$	R^2= 0.940
35°C	$y = -0.87420 + 1.3367e^{-2}x$	R^2= 0.808
40°C	$y = -0.33315 + 6.5177e^{-3}x$	R^2= 0.965
45°C	$y = -0.12869 + 5.8294e^{-3}x$	R^2= 0.976
30% w/v; 1.0 litre/minute; 125 rpm		
30°C	$y = -0.19366 + 3.5107e^{-3}x$	R^2= 0.929
35°C	$y = 0.18481 + 5.1991e^{-3}x$	R^2= 0.963
40°C	$y = -0.13600 + 5.7656e^{-3}x$	R^2= 0.990
45°C	$y = -6.7554e^{-2} + 7.5470e^{-3}x$	R^2= 0.972
35% w/v; 1.0 litre/minute; 125 rpm		
30°C	$y = -0.15183 + 2.1680e^{-3}x$	R^2= 0.895
35°C	$y = 5.8477e^{-2} + 3.0094e^{-3}x$	R^2= 0.972
40°C	$y = -0.14628 + 5.2478e^{-3}x$	R^2= 0.981
45°C	$y = -0.36535 + 6.1935e^{-3}x$	R^2= 0.910

Experimental conditions	Equations derived from plots ln C_o/C_t vs time	
15% w/v; 1.5 litre/minute; 125 rpm		
30°C	$y = 0.10699 + 4.0540e^{-3}x$	R^2= 0.974
35°C	$y = -0.13961 + 6.7826e^{-3}x$	R^2= 0.962
40°C	$y = 0.54693 + 5.6269e^{-3}x$	R^2= 0.823
45°C	$y = 0.47973+ 6.0607e^{-3}x$	R^2= 0.929
20% w/v; 1.5 litre/minute; 125 rpm		
30°C	$y = 0.15183 + 3.9057e^{-3}x$	R^2= 0.950
35°C	$y = -6.6779e^{-2} + 5.6377e^{-3}x$	R^2= 0.976
40°C	$y = 0.42206 + 4.4062e^{-3}x$	R^2= 0.831
45°C	$y = 3.0926e^{-2}+ 3.2228e^{-3}x$	R^2= 0.973
25% w/v; 1.5 litre/minute; 125 rpm		
30°C	$y = -2.0680e^{-3} + 5.0087e^{-3}x$	R^2= 0.944
35°C	$y = 0.22311 + 5.6193e^{-3}x$	R^2= 0.938
40°C	$y = 0.13550 + 6.3461e^{-3}x$	R^2= 0.960
45°C	$y = 0.20454+ 5.6258e^{-3}x$	R^2= 0.893
30% w/v; 1.5 litre/minute; 125 rpm		
30°C	$y = -5.9056e^{-2} + 5.2615e^{-3}x$	R^2= 0.956
35°C	$y = 1.1204e^{-2} + 6.1132e^{-3}x$	R^2= 0.933
40°C	$y = 0.15189 + 5.9657e^{-3}x$	R^2= 0.941
45°C	$y = 0.17262+ 6.8547e^{-3}x$	R^2= 0.921
35% w/v; 1.5 litre/minute; 125 rpm		
30°C	$y = -1.2739e^{-2} + 5.2680e^{-3}x$	R^2= 0.947
35°C	$y = -0.18834 + 5.4940e^{-3}x$	R^2= 0.903
40°C	$y = -6.3382e^{-2} + 5.6215e^{-3}x$	R^2= 0.991
45°C	$y = 0.26843+ 6.3169e^{-3}x$	R^2= 0.864

Experimental conditions	Equations derived from plots ln C_o/C_t vs time	
15% w/v; 1.5 litre/minute; 175 rpm		
30°C	$y = -1.6557e^{-2} + 5.0503e^{-3}x$	R^2= 0.956
35°C	$y = 6.6363e^{-2} + 5.6900e^{-3}x$	R^2= 0.952
40°C	$y = 0.28567 + 5.0813e^{-3}x$	R^2= 0.931
45°C	$y = 0.17929 + 6.9165e^{-3}x$	R^2= 0.920
20% w/v; 1.5 litre/minute; 175 rpm		
30°C	$y = -0.32624 + 5.0255e^{-3}x$	R^2= 0.891
35°C	$y = -6.3766e^{-2} + 5.8829e^{-3}x$	R^2= 0.968
40°C	$y = 0.20302 + 5.7932e^{-3}x$	R^2= 0.928
45°C	$y = 0.34970 + 5.7065e^{-3}x$	R^2= 0.861
25% w/v; 1.5 litre/minute; 175 rpm		
30°C	$y = -0.32392 + 6.1737e^{-3}x$	R^2= 0.943
35°C	$y = 9.2226e^{-2} + 4.9481e^{-3}x$	R^2= 0.912
40°C	$y = 0.16866 + 5.5326e^{-3}x$	R^2= 0.964
45°C	$y = 0.19062 + 5.0261e^{-3}x$	R^2= 0.970
30% w/v; 1.5 litre/minute; 175 rpm		
30°C	$y = -0.21728 + 5.1492e^{-3}x$	R^2= 0.956
35°C	$y = -3.2998e^{-2} + 5.4101e^{-3}x$	R^2= 0.878
40°C	$y = -9.9624e^{-2} + 5.9911e^{-3}x$	R^2= 0.992
45°C	$y = 7.2257e^{-2} + 5.9106e^{-3}x$	R^2= 0.898
35% w/v; 1.5 litre/minute; 175 rpm		
30°C	$y = -0.17358 + 5.1064e^{-3}x$	R^2= 0.935
35°C	$y = -0.14399 + 5.2264e^{-3}x$	R^2= 0.971
40°C	$y = -0.13924 + 6.2440e^{-3}x$	R^2= 0.976
45°C	$y = -1.4985e^{-2} + 7.3739e^{-3}x$	R^2= 0.911

APPENDIX K:

EQUATIONS FROM PLOTS LOG kc VERSUS (1/T)

Experimental conditions	Equations derived from plots ln C_o/C_t vs time	
1.0 litre/minute; 125 rpm		
15%w/v	y = 5.1026 - 2.3271x	R^2= 0.492
20%w/v	y = 3.8465 - 1.9550x	R^2= 1.000
25%w/v	y = 3.9152 - 1.9366x	R^2= 0.801
30%w/v	y = 4.2249 - 2.0175x	R^2= 0.955
35%w/v	y = 7.6233 - 3.1169x	R^2= 0.967
1.5 litre/minute; 125 rpm		
15%w/v	y = 1.44740 - 1.1620x	R^2= 0.979
20%w/v	y = -0.76267 - 0.49868x	R^2= 1.000
25%w/v	y = 0.92899 - 0.97876x	R^2= 1.000
30%w/v	y = -0.13995 - 0.64570x	R^2= 0.837
35%w/v	y = -0.71741 - 0.47478x	R^2= 0.881
1.5 litre/minute; 175 rpm		
15%w/v	y = 0.59095 - 0.87438x	R^2= 0.998
20%w/v	y = -0.33871 - 0.59025x	R^2= 1.000
25%w/v	y = 0.72232 - 0.93252x	R^2= 1.000
30%w/v	y = -0.22752 - 0.62564x	R^2= 0.959
35%w/v	y = 1.22620 - 1.0720x	R^2= 0.921

APPENDIX L:

ACTIVATION ENERGY (E_a) and PRE-EXPONENTIAL RATE (A)

E_a/2.3R	R cal/mol.°K	E_a kcal/mol	E_a KJ/mol	Experiments at 1.0 l/min.,125rpm	log A	A
2.3271	1.986	10.62	44.58	15%w/v, R^2=0.492	5.1026	126648.48
1.9550	1.986	8.93	37.45	20%w/v, R^2=1.000	3.8465	7022.6334
1.9366	1.986	8.85	37.10	25%w/v, R^2=0.801	3.9152	8226.2139
2.0175	1.986	9.22	38.65	30%w/v, R^2=0.955	4.2249	16784.175
3.1169	1.986	14.24	59.71	35%w/v, R^2=0.967	7.6233	42004904.
E_a/2.3R	R cal/mol.°K	E_a kcal/mol	E_a KJ/mol	Experiments at 1.5 l/min.,125rpm	log A	A
1.1620	1.986	5.31	22.26	15%w/v ,R^2=0.979	1.4474	28.0156
0.4987	1.986	2.28	9.55	20%w/v, R^2=1.000	-0.7630	0.1727
0.9788	1.986	4.47	18.75	25%w/v, R^2=1.000	0.9290	8.4916
0.6457	1.986	2.95	12.37	30%w/v, R^2=0.837	-0.1400	0.7245
0.4748	1.986	2.17	9.10	35%w/v, R^2=0.881	-0.7170	0.1917
E_a/2.3R	R cal/mol.°K	E_a kcal/mol	E_a KJ/mol	Experiments at 1.5 l/min.,175 rpm	log A	A
0.8738	1.986	3.99	16.74	15%w/v, R^2=0.998	0.5910	3.8990
0.5902	1.986	2.70	11.31	20%w/v, R^2=0.675	-0.3390	0.4584
0.9325	1.986	4.26	17.86	25%w/v, R^2=1.000	0.7223	5.2762
0.6256	1.986	2.86	11.99	30%w/v, R^2=0.959	-0.2280	0.5922
1.0720	1.986	4.90	20.54	35%w/v, R^2=0.921	1.2262	16.8345

APPENDIX M

PREDICTED PYRITIC SULPHUR REMOVAL (BASED ON KINETIC EQUATION)

Conditions	day	ΣPy removal experiment (mM/l)	ΣPy removal calculated. (mM/l)	ΔPy removal (mM/l)	$\Delta Py/C_o$ (%)
15%w/v	6	5.31	4.31	1.00	8.61
30°C	12	8.43	7.23	1.19	10.27
1.0l/min.	18	8.54	9.22	0.67	5.82
125 rpm	24	9.00	10.56	1.56	13.42
20%w/v	6	6.46	5.43	1.03	8.87
30°C	12	9.39	9.21	0.17	1.49
1.0l/min.	18	13.08	11.84	1.24	10.66
125 rpm	24	13.23	13.67	0.44	3.78
25%w/v	6	10.19	8.63	1.57	13.50
30°C	12	13.08	13.92	0.84	7.24
1.0l/min.	18	18.27	17.17	1.11	9.56
125 rpm	24	18.27	19.15	0.88	7.60
30%w/v	6	3.46	11.17	7.71	66.47
30°C	12	11.54	17.68	6.14	52.95
1.0l/min.	18	21.24	21.48	0.24	2.09
125 rpm	24	22.39	23.69	1.30	11.21
35%w/v	6	4.04	8.58	4.54	39.11
30°C	12	6.19	14.80	8.60	74.17
1.0l/min.	18	16.43	19.31	2.88	24.87
125 rpm	24	22.35	22.59	0.23	2.02
15%w/v	6	4.04	5.27	1.23	10.87
35°C	12	10.73	8.41	2.33	20.58
1.0l/min.	18	12.35	10.28	2.07	18.32
125 rpm	24	12.35	11.39	0.95	8.45
20%w/v	6	8.46	6.43	2.03	17.97
35°C	12	13.54	10.49	3.06	27.04
1.0l/min.	18	16.16	13.04	3.12	27.59
125 rpm	24	16.31	14.65	1.66	14.71
25%w/v	6	12.31	10.05	2.26	19.97
35°C	12	19.24	15.46	3.78	33.42
1.0l/min.	18	20.39	18.36	2.03	17.93
125 rpm	24	21.74	19.93	1.81	16.03
30%w/v	6	15.70	13.03	2.67	23.60
35°C	12	22.62	19.55	3.07	27.19
1.0l/min.	18	24.01	22.81	1.19	10.57
125 rpm	24	24.70	24.44	0.25	2.25
35%w/v	6	14.81	11.43	3.38	29.89
35°C	12	16.70	18.57	1.88	16.60
1.0l/min.	18	22.35	23.03	0.68	5.98

125 rpm	24	25.58	25.81	0.23	2.00
15%w/v	6	8.66	6.86	1.80	15.01
40°C	12	11.31	10.32	0.99	8.28
1.0l/min.	18	11.77	12.07	0.29	2.45
125 rpm	24	13.04	12.95	0.09	0.77
20%w/v	6	10.46	8.16	2.30	19.18
40°C	12	14.77	12.72	2.06	17.13
1.0l/min.	18	15.39	15.26	0.13	1.07
125 rpm	24	16.77	16.68	0.09	0.79
25%w/v	6	7.31	12.52	5.21	43.44
40°C	12	16.54	18.25	1.71	14.24
1.0l/min.	18	20.97	20.87	0.10	0.80
125 rpm	24	22.51	22.07	0.44	3.63
30%w/v	6	11.54	16.22	4.68	38.97
40°C	12	21.01	22.94	1.94	16.13
1.0l/min.	18	25.39	25.72	0.33	2.76
125 rpm	24	26.55	26.88	0.34	2.81
35%w/v	6	14.27	16.00	1.73	14.41
40°C	12	21.54	24.08	2.54	21.15
1.0l/min.	18	28.55	28.16	0.39	3.21
125 rpm	24	30.70	30.22	0.48	4.00
15%w/v	6	7.96	8.53	0.56	4.51
45°C	12	13.04	12.02	1.03	8.21
1.0l/min.	18	13.50	13.44	0.06	0.49
125 rpm	24	13.50	14.03	0.52	4.18
20%w/v	6	9.69	9.98	0.28	2.27
45°C	12	10.46	14.78	4.32	34.54
1.0l/min.	18	12.16	17.09	4.94	39.49
125 rpm	24	16.00	18.21	2.20	17.62
25%w/v	6	7.89	15.00	7.12	56.92
45°C	12	20.01	20.64	0.64	5.10
1.0l/min.	18	21.74	22.76	1.03	8.21
125 rpm	24	23.08	23.56	0.48	3.82
30%w/v	6	13.62	19.36	5.74	45.93
45°C	12	26.55	25.73	0.82	6.54
1.0l/min.	18	27.70	27.82	0.12	0.99
125 rpm	24	28.39	28.51	0.12	0.96
35%w/v	6	12.93	21.02	8.10	64.78
45°C	12	20.47	28.99	8.52	68.20
1.0l/min.	18	28.81	31.88	3.06	24.51
125 rpm	24	32.85	32.99	0.14	1.10
15%w/v	6	6.12	5.54	0.57	5.36
30°C	12	9.69	8.60	1.10	10.24
1.5l/min.	18	10.27	10.28	0.01	0.10
125 rpm	24	11.20	11.21	0.02	0.14
20%w/v	6	8.31	7.11	1.20	11.26
30°C	12	13.23	11.15	2.09	19.52
1.5l/min.	18	13.70	13.44	0.25	2.37

125 rpm	24	14.77	14.75	0.02	0.23
25%w/v	6	7.50	10.62	3.12	29.13
30°C	12	16.73	15.76	0.98	9.12
1.5l/min.	18	18.85	18.25	0.60	5.65
125 rpm	24	19.04	19.45	0.41	3.81
30%w/v	6	9.23	13.33	4.10	38.29
30°C	12	19.16	19.47	0.31	2.88
1.5l/min.	18	22.85	22.29	0.56	5.24
125 rpm	24	23.08	23.59	0.51	4.75
35%w/v	6	10.77	15.22	4.45	41.55
30°C	12	23.70	22.40	1.30	12.16
1.5l/min.	18	26.66	25.78	0.88	8.18
125 rpm	24	26.93	27.38	0.45	4.23
15%w/v	6	6.35	6.54	0.19	1.71
35°C	12	11.43	9.83	1.59	13.98
1.5l/min.	18	11.89	11.49	0.40	3.52
125 rpm	24	12.93	12.32	0.61	5.33
20%w/v	6	6.62	7.92	1.30	11.41
35°C	12	14.77	12.26	2.51	22.03
1.5l/min.	18	15.85	14.64	1.21	10.58
125 rpm	24	16.77	15.95	0.82	7.21
25%w/v	6	13.46	12.26	1.21	10.58
35°C	12	19.81	17.66	2.15	18.84
1.5l/min.	18	20.58	20.05	0.53	4.67
125 rpm	24	20.97	21.10	0.13	1.17
30%w/v	6	11.54	14.95	3.41	29.93
35°C	12	22.85	21.41	1.44	12.67
1.5l/min.	18	25.16	24.19	0.97	8.47
125 rpm	24	25.16	25.40	0.24	2.07
35%w/v	6	11.31	16.86	5.55	48.66
35°C	12	18.58	24.46	5.88	51.55
1.5l/min.	18	28.81	27.89	0.93	8.15
125 rpm	24	28.81	29.43	0.62	5.40
15%w/v	6	11.43	8.13	3.30	25.55
40°C	12	14.20	11.82	2.37	18.41
1.0l/min.	18	14.20	13.50	0.70	5.42
125 rpm	24	14.31	14.26	0.05	0.42
20%w/v	6	13.54	9.35	4.19	32.47
40°C	12	18.00	14.30	3.70	28.72
1.0l/min.	18	18.00	16.92	1.09	8.45
125 rpm	24	18.31	18.30	0.01	0.11
25%w/v	6	14.62	14.92	0.30	2.36
40°C	12	22.51	20.87	1.64	12.68
1.0l/min.	18	23.66	23.24	0.42	3.25
125 rpm	24	24.04	24.18	0.14	1.09
30%w/v	6	16.62	17.76	1.14	8.85
40°C	12	27.01	24.93	2.08	16.10
1.0l/min.	18	28.16	27.82	0.34	2.62

125 rpm	24	28.62	28.99	0.37	2.85
35%w/v	6	16.16	19.79	3.63	28.13
40°C	12	28.28	28.30	0.03	0.21
1.0l/min.	18	30.97	31.97	1.00	7.75
125 rpm	24	33.39	33.55	0.15	1.20
15%w/v	6	12.23	8.72	3.52	27.68
45°C	12	13.50	12.25	1.25	9.85
1.5l/min.	18	13.97	13.68	0.28	2.21
125 rpm	24	14.31	14.26	0.05	0.37
20%w/v	6	7.08	9.59	2.51	19.78
45°C	12	13.39	14.47	1.09	8.55
1.5l/min.	18	14.16	16.96	2.80	22.07
125 rpm	24	16.62	18.23	1.61	12.65
25%w/v	6	13.08	15.71	2.63	20.71
45°C	12	22.51	21.32	1.19	9.35
1.5l/min.	18	22.89	23.32	0.43	3.38
125 rpm	24	23.28	24.03	0.76	5.97
30%w/v	6	16.85	18.29	1.44	11.34
45°C	12	27.70	25.17	2.53	19.91
1.5l/min.	18	28.16	27.76	0.40	3.16
125 rpm	24	28.62	28.33	0.29	2.32
35%w/v	6	19.93	20.16	0.23	1.84
45°C	12	32.59	28.44	4.15	32.66
1.5l/min.	18	32.59	31.89	0.69	5.45
125 rpm	24	33.12	33.23	0.11	0.83
15%w/v	6	4.15	6.31	2.15	20.92
30°C	12	9.81	9.27	0.54	5.24
1.5l/min.	18	10.73	10.66	0.07	0.71
175 rpm	24	11.08	11.31	0.23	2.26
20%w/v	6	4.00	8.35	4.35	42.19
30°C	12	6.00	12.30	6.30	61.13
1.5l/min.	18	14.00	14.17	0.17	1.60
175 rpm	24	14.77	15.05	0.28	2.73
25%w/v	6	5.19	9.37	4.18	40.54
30°C	12	12.70	14.31	1.61	15.65
1.5l/min.	18	18.47	16.91	1.56	15.11
175 rpm	24	19.04	18.28	0.76	7.39
30%w/v	6	2.54	12.42	9.88	95.90
30°C	12	17.77	18.35	0.58	5.60
1.5l/min.	18	21.01	21.19	0.18	1.75
175 rpm	24	22.16	22.54	0.38	3.70
35%w/v	6	4.85	14.09	9.24	89.70
30°C	12	20.20	21.02	0.82	7.99
1.5l/min.	18	25.31	24.43	0.88	8.56
175 rpm	24	25.58	26.11	0.53	5.13
15%w/v	6	6.35	7.62	1.28	10.99
35°C	12	11.89	10.91	0.98	8.46
1.5l/min.	18	12.35	12.32	0.03	0.25

175 rpm	24	12.81	12.93	0.12	1.02
20%w/v	6	7.39	9.86	2.48	21.36
35°C	12	15.39	14.28	1.11	9.58
1.5l/min.	18	16.00	16.25	0.25	2.13
175 rpm	24	17.24	17.13	0.10	0.86
25%w/v	6	9.43	11.43	2.01	17.32
35°C	12	19.04	17.01	2.04	17.54
1.5l/min.	18	20.58	19.73	0.86	7.39
175 rpm	24	20.58	21.05	0.47	4.03
30%w/v	6	5.54	14.72	9.18	79.13
35°C	12	23.31	21.35	1.97	16.94
1.5l/min.	18	24.93	24.33	0.60	5.14
175 rpm	24	24.93	25.68	0.75	6.46
35%w/v	6	7.54	17.33	9.79	84.38
35°C	12	24.51	25.05	0.54	4.65
1.5l/min.	18	27.74	28.48	0.74	6.41
175 rpm	24	29.35	30.01	0.66	5.67
15%w/v	6	10.50	9.60	0.90	6.57
40°C	12	13.85	13.37	0.48	3.48
1.5l/min.	18	14.77	14.85	0.08	0.59
175 rpm	24	14.89	15.43	0.55	3.99
20%w/v	6	12.46	12.19	0.28	2.03
40°C	12	19.08	17.33	1.75	12.80
1.5l/min.	18	20.01	19.50	0.51	3.71
175 rpm	24	20.16	20.41	0.25	1.85
25%w/v	6	15.77	14.55	1.23	8.96
40°C	12	23.28	21.06	2.21	16.15
1.5l/min.	18	24.43	23.98	0.45	3.27
175 rpm	24	25.20	25.29	0.09	0.66
30%w/v	6	13.62	18.22	4.60	33.59
40°C	12	25.85	25.94	0.08	0.61
1.5l/min.	18	29.08	29.20	0.12	0.86
175 rpm	24	30.47	30.58	0.12	0.84
35%w/v	6	14.00	22.18	8.18	59.68
40°C	12	29.89	31.02	1.13	8.26
1.5l/min.	18	34.74	34.55	0.19	1.38
175 rpm	24	35.55	35.96	0.41	2.99
15%w/v	6	8.19	9.07	0.88	7.21
45°C	12	13.27	12.30	0.97	7.97
1.5l/min.	18	13.62	13.45	0.17	1.41
175 rpm	24	13.73	13.86	0.12	0.99
20%w/v	6	12.16	11.32	0.84	6.87
45°C	12	17.70	15.81	1.88	15.45
1.5l/min.	18	17.70	17.60	0.10	0.83
175 rpm	24	18.00	18.30	0.30	2.45
25%w/v	6	14.62	13.87	0.75	6.11
45°C	12	19.81	19.55	0.27	2.18
1.5l/min.	18	21.54	21.87	0.32	2.64

175 rpm	24	22.12	22.81	0.69	5.68
30%w/v	6	11.54	16.98	5.44	44.59
45°C	12	25.39	23.72	1.67	13.68
1.5l/min.	18	26.78	26.40	0.38	3.09
175 rpm	24	26.78	27.46	0.68	5.61
35%w/v	6	13.20	21.25	8.05	65.99
45°C	12	30.43	28.75	1.68	13.77
1.5l/min.	18	32.05	31.40	0.64	5.28
175 rpm	24	32.05	32.34	0.29	2.39

APPENDIX N:

CALCULATION OF PYRITIC SULPHUR REMOVAL (BASED ON EXPERIMENTAL DATA)

% Py mv	coal (gr)	time hour	Pymv	ΣPymv (M/l)	Vpy (M/l.h)e-4	Condition	C_o (M/l)	$C_t = C_o - \Sigma Pymv$ (M/l)	$\ln C_o/C_t$
0.46	180	144	0.63692308	0.00530902	0.36868191	15%w/v	0.01339	0.00808098	0.50499495
0.73	180	288	0.37384615	0.00842518	0.29254108	30°C	0.01339	0.00496482	0.99213176
0.74	180	432	0.01384615	0.0085406	0.197699	1.0l/min.	0.01339	0.0048494	1.01565249
0.78	180	576	0.05538462	0.00900225	0.15628907	125 rpm	0.01339	0.00438775	1.11569172
0.42	240	144	0.77538462	0.00646315	0.44883016	20%w/v	0.01785	0.01138685	0.4495447
0.61	240	288	0.35076923	0.00938696	0.32593618	30°C	0.01785	0.00846304	0.74629531
0.85	240	432	0.44307692	0.01308019	0.30278225	1.0l/min.	0.01785	0.00476981	1.31969769
0.86	240	576	0.01846154	0.01323408	0.22975829	125 rpm	0.01785	0.00461592	1.35249182
0.53	300	144	1.22307692	0.01019486	0.70797614	25%w/v	0.02231	0.01211514	0.6105788
0.68	300	288	0.34615385	0.01308019	0.45417337	30°C	0.02231	0.00922981	0.88259688
0.95	300	432	0.62307692	0.0182738	0.42300461	1.0l/min.	0.02231	0.0040362	1.70973116
0.95	300	576	0	0.0182738	0.31725346	125 rpm	0.02231	0.0040362	1.70973116
0.15	360	144	0.41538462	0.0034624	0.24044473	30%w/v	0.02678	0.0233176	0.13844707
0.5	360	288	0.96923077	0.01154135	0.40074121	30°C	0.02678	0.01523865	0.56382017
0.92	360	432	1.16307692	0.02123608	0.49157589	1.0l/min.	0.02678	0.00554392	1.57495319
0.97	360	576	0.13846154	0.02239021	0.38871897	125 rpm	0.02678	0.00438979	1.80837462
0.15	420	144	0.48461538	0.00403947	0.28051885	35%w/v	0.03124	0.02720053	0.13846292
0.23	420	288	0.25846154	0.00619386	0.21506445	30°C	0.03124	0.02504614	0.22097945
0.61	420	432	1.22769231	0.01642718	0.38025888	1.0l/min.	0.03124	0.01481282	0.74620655
0.83	420	576	0.71076923	0.02235174	0.38805107	125 rpm	0.03124	0.00888826	1.25696822
0.35	180	144	0.48461538	0.00403947	0.28051885	15%w/v	0.01304	0.00900053	0.37073825
0.93	180	288	0.80307692	0.01073345	0.37268933	35°C	0.01304	0.00230655	1.73226978
1.07	180	432	0.19384615	0.01234924	0.28586206	1.0l/min.	0.01304	0.00069076	2.93798607
1.07	180	576	0	0.01234924	0.21439655	125 rpm	0.01304	0.00069076	2.93798607
0.55	240	144	1.01538462	0.00846365	0.58775378	20%w/v	0.01739	0.00892635	0.66688824
0.88	240	288	0.60923077	0.01354185	0.47020302	35°C	0.01739	0.00384815	1.50830204
1.05	240	432	0.31384615	0.01615789	0.37402513	1.0l/min.	0.01739	0.00123211	2.64716363
1.06	240	576	0.01846154	0.01631177	0.28319046	125 rpm	0.01739	0.00107823	2.78057475
0.64	300	144	1.47692308	0.01231077	0.85491458	25%w/v	0.02174	0.00942923	0.83533944
1	300	288	0.83076923	0.01923558	0.66790202	35°C	0.02174	0.00250442	2.16109596
1.06	300	432	0.13846154	0.02038971	0.47198409	1.0l/min.	0.02174	0.00135029	2.77883658
1.13	300	576	0.16153846	0.0217362	0.37736464	125 rpm	0.02174	3.7967E-06	8.65277204
0.68	360	144	1.88307692	0.01569623	1.09001609	30%w/v	0.02608	0.01038377	0.9209249
0.98	360	288	0.83076923	0.02262104	0.78545277	35°C	0.02608	0.00345896	2.02020074
1.04	360	432	0.16615385	0.024006	0.55569448	1.0l/min.	0.02608	0.002074	2.53169035
1.07	360	576	0.08307692	0.02469848	0.4287931	125 rpm	0.02608	0.00138152	2.93798607
0.55	420	144	1.77692308	0.0148114	1.02856911	35%w/v	0.03043	0.0156186	0.66696614
0.62	420	288	0.22615385	0.01669648	0.57973895	35°C	0.03043	0.01373352	0.79558954
0.83	420	432	0.67846154	0.02235174	0.51740143	1.0l/min.	0.03043	0.00807826	1.32625268
0.95	420	576	0.38769231	0.02558332	0.44415484	125 rpm	0.03043	0.00484668	1.8371348

0.12	300	144	0.27692308	0.00230827	0.16029648	25%w/v	0.02174	0.01943173	0.11224655
0.23	300	288	0.25384615	0.00442418	0.15361746	35°C	0.02174	0.01731582	0.22753352
0.49	300	432	0.6	0.00942543	0.21818133	1.0l/min.	0.02174	0.01231457	0.56837103
1.07	300	576	0.18461538	0.02250563	0.39072268	125 rpm	0.02174	0.00207783	0.89766532
0.75	180	144	1.03846154	0.00865601	0.60111182	15%w/v	0.01385	0.00519399	0.98078307
0.98	180	288	0.31846154	0.01131052	0.39272639	40°C	0.01385	0.00253948	1.69632587
1.02	180	432	0.05538462	0.01177217	0.27250402	1.0l/min.	0.01385	0.00207783	1.89696299
1.13	180	576	0.15230769	0.01304172	0.22641878	125 rpm	0.01385	0.00080828	2.84113441
0.68	240	144	1.25538462	0.01046415	0.7266774	20%w/v	0.01847	0.00800585	0.83597583
0.96	240	288	0.51692308	0.01477292	0.51294875	40°C	0.01847	0.00369708	1.60860556
1	240	432	0.07384615	0.01538846	0.35621441	1.0l/min.	0.01847	0.00308154	1.79071913
1.09	240	576	0.16615385	0.01677342	0.29120528	125 rpm	0.01847	0.00169658	2.38753577
0.38	300	144	0.87692308	0.00730952	0.50760553	25%w/v	0.02308	0.01577048	0.38082658
0.86	300	288	1.10769231	0.0165426	0.57439574	40°C	0.02308	0.0065374	1.26142648
1.09	300	432	0.53076923	0.02096678	0.48534213	1.0l/min.	0.02308	0.00211322	2.39075367
1.17	300	576	0.18461538	0.02250563	0.39072268	125 rpm	0.02308	0.00057437	3.69344168
0.5	360	144	1.38461538	0.01154135	0.80148242	30%w/v	0.0277	0.01615865	0.53897671
0.91	360	288	1.13538462	0.02100525	0.729349	40°C	0.0277	0.00669475	1.42010897
1.1	360	432	0.52615385	0.02539096	0.58775378	1.0l/min.	0.0277	0.00230904	2.48460191
1.15	360	576	0.13846154	0.0265451	0.46085239	125 rpm	0.0277	0.0011549	3.17741676
0.53	420	144	1.71230769	0.0142728	0.9911666	35%w/v	0.03232	0.0180472	0.58269563
0.8	420	288	0.87230769	0.02154385	0.74805026	40°C	0.03232	0.01077615	1.09835064
1.06	420	432	0.84	0.0285456	0.66077773	1.0l/min.	0.03232	0.0037744	2.14744426
1.14	420	576	0.25846154	0.03069998	0.53298581	125 rpm	0.03232	0.00162002	2.9932494
0.69	180	144	0.95538462	0.00796353	0.55302287	15%w/v	0.01443	0.00646647	0.80267891
1.13	180	288	0.60923077	0.01304172	0.45283757	45°C	0.01443	0.00138828	2.34124522
1.17	180	432	0.05538462	0.01350338	0.31257814	1.0l/min.	0.01443	0.00092662	2.74551661
1.17	180	576	0	0.01350338	0.23443361	125 rpm	0.01443	0.00092662	2.74551661
0.63	240	144	1.16307692	0.00969473	0.67324523	20%w/v	0.01924	0.00954527	0.70094585
0.68	240	288	0.09230769	0.01046415	0.3633387	45°C	0.01924	0.00877585	0.78498833
0.79	240	432	0.20307692	0.01215689	0.28140938	1.0l/min.	0.01924	0.00708311	0.99927772
1.04	240	576	0.46153846	0.016004	0.27784724	125 rpm	0.01924	0.003236	1.78265376
0.41	300	144	0.94615385	0.00788659	0.54767965	25%w/v	0.02404	0.01615341	0.39758775
1.04	300	288	1.45384615	0.020005	0.6946181	45°C	0.02404	0.004035	1.78471312
1.13	300	432	0.20769231	0.0217362	0.50315285	1.0l/min.	0.02404	0.0023038	2.3451606
1.2	300	576	0.16153846	0.02308269	0.40074121	125 rpm	0.02404	0.00095731	3.22335104
0.59	360	144	1.63384615	0.01361879	0.94574926	30%w/v	0.02885	0.01523121	0.63876334
1.15	360	288	1.55076923	0.0265451	0.92170479	45°C	0.02885	0.0023049	2.52707175
1.2	360	432	0.13846154	0.02769923	0.64118594	1.0l/min.	0.02885	0.00115077	3.22168088
1.23	360	576	0.08307692	0.02839171	0.49291169	125 rpm	0.02885	0.00045829	4.14237033
0.48	420	144	1.55076923	0.01292631	0.89766031	35%w/v	0.03366	0.02073369	0.4845502
0.76	420	288	0.90461538	0.02046666	0.71064775	45°C	0.03366	0.01319334	0.93659766
1.07	420	432	1.00153846	0.0288149	0.66701148	1.0l/min.	0.03366	0.0048451	1.93834148
1.22	420	576	0.48461538	0.03285437	0.57038832	125 rpm	0.03366	0.00080563	3.73243771
1.06	180	144	1.46769231	0.01223383	0.84957137	15%w/v	0.01466	0.00242617	1.79880786
1.17	180	288	0.15230769	0.01350338	0.46886722	45°C	0.01466	0.00115662	2.53961714

1.21	180	432	0.05538462	0.01396503	0.32326458	1.5l/min.	0.01466	0.00069497	3.04900889
1.24	180	576	0.04153846	0.01431127	0.24845955	125 rpm	0.01466	0.00034873	3.73858035
0.46	240	144	0.84923077	0.00707869	0.49157589	20%w/v	0.01954	0.01246131	0.44983522
0.87	240	288	0.75692308	0.01338796	0.4648598	45°C	0.01954	0.00615204	1.1556803
0.92	240	432	0.09230769	0.01415739	0.32771726	1.5l/min.	0.01954	0.00538261	1.28928942
1.08	240	576	0.29538462	0.01661954	0.28853367	125 rpm	0.01954	0.00292046	1.90072234
0.68	300	144	1.56923077	0.01308019	0.90834674	25%w/v	0.02443	0.01134981	0.76661116
1.17	300	288	1.13076923	0.02250563	0.78144536	45°C	0.02443	0.00192437	2.54121138
1.19	300	432	0.04615385	0.02289034	0.52986893	1.5l/min.	0.02443	0.00153966	2.76424895
1.21	300	576	0.04615385	0.02327505	0.40408072	125 rpm	0.02443	0.00115495	3.05175443
0.73	360	144	2.02153846	0.01685037	1.17016434	30%w/v	0.02932	0.01246963	0.8549735
1.2	360	288	1.30153846	0.02769923	0.96177891	45°C	0.02932	0.00162077	2.89537007
1.22	360	432	0.05538462	0.02816089	0.65187237	1.5l/min.	0.02932	0.00115911	3.23061428
1.24	360	576	0.05538462	0.02862254	0.4969191	125 rpm	0.02932	0.00069746	3.73858035
0.74	420	144	2.39076923	0.01992806	1.38389298	35%w/v	0.0342	0.01427194	0.8739302
1.21	420	288	1.51846154	0.03258507	1.13142602	45°C	0.0342	0.00161493	3.05293363
1.21	420	432	0	0.03258507	0.75428401	1.5l/min.	0.0342	0.00161493	3.05293363
1.23	420	576	0.06461538	0.03312367	0.57506364	125 rpm	0.0342	0.00107633	3.45866439
0.99	180	144	1.37076923	0.01142593	0.7934676	15%w/v	0.01489	0.00346407	1.45824663
1.23	180	288	0.33230769	0.01419586	0.49291169	40°C	0.01489	0.00069414	3.06576664
1.23	180	432	0	0.01419586	0.32860779	1.0l/min.	0.01489	0.00069414	3.06576664
1.24	180	576	0.01384615	0.01431127	0.24845955	125 rpm	0.01489	0.00057873	3.24760929
0.88	240	144	1.62461538	0.01354185	0.94040604	20%w/v	0.01985	0.00630815	1.14636108
1.17	240	288	0.53538462	0.0180045	0.62515629	40°C	0.01985	0.0018455	2.37545437
1.17	240	432	0	0.0180045	0.41677086	1.0l/min.	0.01985	0.0018455	2.37545437
1.19	240	576	0.03692308	0.01831227	0.31792136	125 rpm	0.01985	0.00153773	2.55789695
0.76	300	144	1.75384615	0.01461904	1.01521107	25%w/v	0.02481	0.01019096	0.88974568
1.17	300	288	0.94615385	0.02250563	0.78144536	40°C	0.02481	0.00230437	2.37643792
1.23	300	432	0.13846154	0.02365976	0.54767965	1.0l/min.	0.02481	0.00115024	3.07127713
1.25	300	576	0.04615385	0.02404447	0.41743876	125 rpm	0.02481	0.00076553	3.47843714
0.72	360	144	1.99384615	0.01661954	1.15413469	30%w/v	0.02978	0.01316046	0.81662011
1.17	360	288	1.24615385	0.02700675	0.93773443	40°C	0.02978	0.00277325	2.37381772
1.22	360	432	0.13846154	0.02816089	0.65187237	1.0l/min.	0.02978	0.00161911	2.91195817
1.24	360	576	0.05538462	0.02862254	0.4969191	125 rpm	0.02978	0.00115746	3.24760929
0.6	420	144	1.93846154	0.01615789	1.12207539	35%w/v	0.03474	0.01858211	0.62569224
1.05	420	288	1.45384615	0.0282763	0.98181597	40°C	0.03474	0.0064637	1.68168983
1.15	420	432	0.32307692	0.03096928	0.7168815	1.0l/min.	0.03474	0.00377072	2.220626
1.24	420	576	0.29076923	0.03339296	0.57973895	125 rpm	0.03474	0.00134704	3.24998486
0.91	180	144	1.26	0.01050263	0.729349	15%w/v	0.01581	0.00530737	1.09154541
1.2	180	288	0.40153846	0.01384962	0.48088945	40°C	0.01581	0.00196038	2.08750241
1.28	180	432	0.11076923	0.01477292	0.34196583	1.5l/min.	0.01581	0.00103708	2.72423744
1.29	180	576	0.01384615	0.01488834	0.25847808	175 rpm	0.01581	0.00092166	2.84221879
0.81	240	144	1.49538462	0.01246465	0.86560102	20%w/v	0.02108	0.00861535	0.89477976
1.24	240	288	0.79384615	0.01908169	0.6625588	40°C	0.02108	0.00199831	2.35602465
1.3	240	432	0.11076923	0.020005	0.46307873	1.5l/min.	0.02108	0.001075	2.97600523
1.31	240	576	0.01846154	0.02015889	0.34998066	175 rpm	0.02108	0.00092111	3.13049606
0.82	300	144	1.89230769	0.01577317	1.09535931	25%w/v	0.02635	0.01057683	0.9128029
1.21	300	288	0.9	0.02327505	0.80816144	40°C	0.02635	0.00307495	2.14817948

1.27	300	432	0.13846154	0.02442918	0.56549038	1.5l/min.	0.02635	0.00192082	2.61871829
1.31	300	576	0.09230769	0.02519861	0.43747582	175 rpm	0.02635	0.00115139	3.13049606
0.59	360	144	1.63384615	0.01361879	0.94574926	30%w/v	0.0316	0.01798121	0.56382976
1.12	360	288	1.46769231	0.02585262	0.89766031	40°C	0.0316	0.00574738	1.7044125
1.26	360	432	0.38769231	0.02908419	0.67324523	1.5l/min.	0.0316	0.00251581	2.53056394
1.32	360	576	0.16615385	0.03046916	0.5289784	175 rpm	0.0316	0.00113084	3.33019264
0.52	420	144	1.68	0.0140035	0.97246534	35%w/v	0.03689	0.0228865	0.47739333
1.11	420	288	1.90615385	0.02989209	1.03791974	40°C	0.03689	0.00699791	1.66232875
1.29	420	432	0.58153846	0.03473945	0.80415403	1.5l/min.	0.03689	0.00215055	2.84221879
1.32	420	576	0.09692308	0.03554735	0.61714146	175 rpm	0.03689	0.00134265	3.31329403
0.71	180	144	0.98307692	0.00819436	0.56905252	15%w/v	0.01408	0.00588564	0.87223923
1.15	180	288	0.60923077	0.01327255	0.46085239	45°C	0.01408	0.00080745	2.85862814
1.18	180	432	0.04153846	0.01361879	0.31524975	1.5l/min.	0.01408	0.00046121	3.41865567
1.19	180	576	0.01384615	0.0137342	0.23844102	175 rpm	0.01408	0.0003458	3.7066581
0.79	240	144	1.45846154	0.01215689	0.84422815	20%w/v	0.01877	0.00661311	1.04320511
1.15	240	288	0.66461538	0.01769673	0.61446986	45°C	0.01877	0.00107327	2.86155153
1.15	240	432	0	0.01769673	0.40964657	1.5l/min.	0.01877	0.00107327	2.86155153
1.17	240	576	0.03692308	0.0180045	0.31257814	175 rpm	0.01877	0.0007655	3.19948738
0.76	300	144	1.75384615	0.01461904	1.01521107	25%w/v	0.02347	0.00885096	0.97519701
1.03	300	288	0.62307692	0.01981265	0.68793908	45°C	0.02347	0.00365735	1.85898293
1.12	300	432	0.20769231	0.02154385	0.49870017	1.5l/min.	0.02347	0.00192615	2.50019852
1.15	300	576	0.06923077	0.02212091	0.38404366	175 rpm	0.02347	0.00134909	2.85629631
0.5	360	144	1.38461538	0.01154135	0.80148242	30%w/v	0.02816	0.01661865	0.52737678
1.1	360	288	1.66153846	0.02539096	0.88163066	45°C	0.02816	0.00276904	2.31940297
1.16	360	432	0.16615385	0.02677592	0.61981307	1.5l/min.	0.02816	0.00138408	3.0128703
1.16	360	576	0	0.02677592	0.4648598	175 rpm	0.02816	0.00138408	3.0128703
0.49	420	144	1.58307692	0.01319561	0.91636157	35%w/v	0.03285	0.01965439	0.51365085
1.13	420	288	2.06769231	0.03043068	1.05662099	45°C	0.03285	0.00241932	2.60846713
1.19	420	432	0.19384615	0.03204647	0.74181651	1.5l/min.	0.03285	0.00080353	3.71069643
1.19	420	576	0	0.03204647	0.55636238	175 rpm	0.03285	0.00080353	3.71069643
0.55	180	144	0.76153846	0.00634774	0.44081533	15%w/v	0.01339	0.00704226	0.64257913
1.03	180	288	0.66461538	0.01188759	0.41276345	35°C	0.01339	0.00150241	2.18743586
1.07	180	432	0.05538462	0.01234924	0.28586206	1.5l/min.	0.01339	0.00104076	2.55455806
1.11	180	576	0.05538462	0.0128109	0.22241137	175 rpm	0.01339	0.0005791	3.14077968
0.48	240	144	0.88615385	0.00738646	0.51294875	15%w/v	0.01785	0.01046354	0.53410687
1	240	288	0.96	0.01538846	0.53432161	35°C	0.01785	0.00246154	1.98121735
1.04	240	432	0.07384615	0.016004	0.37046299	1.5l/min.	0.01785	0.001846	2.26898291
1.12	240	576	0.14769231	0.01723508	0.2992201	175 rpm	0.01785	0.00061492	3.36826336
0.49	300	144	1.13076923	0.00942543	0.65454398	25%w/v	0.02231	0.01288457	0.54900479
0.99	300	288	1.15384615	0.01904322	0.661223	35°C	0.02231	0.00326678	1.92123094
1.07	300	432	0.18461538	0.02058207	0.47643677	1.5l/min.	0.02231	0.00172793	2.55811003
1.07	300	576	0	0.02058207	0.35732758	175 rpm	0.02231	0.00172793	2.55811003
0.24	360	144	0.66461538	0.00553985	0.38471156	30%w/v	0.02678	0.02124015	0.23176192
1.01	360	288	2.13230769	0.02331352	0.80949725	35°C	0.02678	0.00346648	2.04451587
1.08	360	432	0.19384615	0.02492931	0.57706734	1.5l/min.	0.02678	0.00185069	2.67209639
1.08	360	576	0	0.02492931	0.43280051	175 rpm	0.02678	0.00185069	2.67209639
0.28	420	144	0.90461538	0.00754035	0.52363518	35%w/v	0.03124	0.02369965	0.2762389
0.91	420	288	2.03538462	0.02450613	0.85090717	35°C	0.03124	0.00673387	1.53454879

1.03	420	432	0.38769231	0.0277377	0.64207647	1.5l/min.	0.03124	0.0035023	2.18828047
1.09	420	576	0.19384615	0.02935349	0.50960924	175 rpm	0.03124	0.00188651	2.80697194
0.36	180	144	0.49846154	0.00415488	0.28853367	15%w/v	0.01189	0.00773512	0.42992734
0.85	180	288	0.67846154	0.00981014	0.34063003	30°C	0.01189	0.00207986	1.74339946
0.93	180	432	0.11076923	0.01073345	0.24845955	1.5l/min.	0.01189	0.00115655	2.33025852
0.96	180	576	0.04153846	0.01107969	0.19235578	175 rpm	0.01189	0.00081031	2.6860398
0.26	240	144	0.48	0.004001	0.27784724	20%w/v	0.01585	0.011849	0.29092605
0.39	240	288	0.24	0.0060015	0.20838543	30°C	0.01585	0.0098485	0.47585038
0.91	240	432	0.96	0.0140035	0.32415511	1.5l/min.	0.01585	0.0018465	2.14987802
0.96	240	576	0.09230769	0.01477292	0.25647438	175 rpm	0.01585	0.00107708	2.68891954
0.27	300	144	0.62307692	0.00519361	0.36066709	25%w/v	0.01981	0.01461639	0.30404309
0.66	300	288	0.9	0.01269548	0.44081533	30°C	0.01981	0.00711452	1.02404931
0.96	300	432	0.69230769	0.01846616	0.42745729	1.5l/min.	0.01981	0.00134384	2.69065195
0.99	300	576	0.06923077	0.01904322	0.3306115	175 rpm	0.01981	0.00076678	3.25174527
0.11	360	144	0.30461538	0.0025391	0.17632613	30%w/v	0.02378	0.0212409	0.11291615
0.77	360	288	1.82769231	0.01777367	0.61714146	30°C	0.02378	0.00600633	1.37603168
0.91	360	432	0.38769231	0.02100525	0.48623267	1.5l/min.	0.02378	0.00277475	2.14828471
0.96	360	576	0.13846154	0.02215939	0.38471156	175 rpm	0.02378	0.00162061	2.6860398
0.18	420	144	0.58153846	0.00484737	0.33662262	35%w/v	0.02774	0.02289263	0.1920602
0.75	420	288	1.84153846	0.02019736	0.70129712	30°C	0.02774	0.00754264	1.30230277
0.94	420	432	0.61384615	0.02531402	0.5859727	1.5l/min.	0.02774	0.00242598	2.43664018
0.95	420	576	0.03230769	0.02558332	0.44415484	175 rpm	0.02774	0.00215668	2.55430491
0.53	180	144	0.73384615	0.00611691	0.42478568	15%w/v	0.01235	0.00623309	0.68378448
0.84	180	288	0.42923077	0.00969473	0.33662262	30°C	0.01235	0.00265527	1.53711024
0.89	180	432	0.06923077	0.0102718	0.23777312	1.5l/min.	0.01235	0.0020782	1.78215331
0.97	180	576	0.11076923	0.01119511	0.19435949	125 rpm	0.01235	0.00115489	2.3696479
0.54	240	144	0.99692308	0.00830977	0.57706734	20%w/v	0.01647	0.00816023	0.70226816
0.86	240	288	0.59076923	0.01323408	0.45951659	30°C	0.01647	0.00323592	1.62722657
0.89	240	432	0.05538462	0.01369573	0.31703082	1.5l/min.	0.01647	0.00277427	1.78115348
0.96	240	576	0.12923077	0.01477292	0.25647438	125 rpm	0.01647	0.00169708	2.27263377
0.39	300	144	0.9	0.00750188	0.52096357	25%w/v	0.02058	0.01307812	0.45337878
0.87	300	288	1.10769231	0.01673495	0.58107476	30°C	0.02058	0.00384505	1.6775339
0.98	300	432	0.25384615	0.01885087	0.43636265	1.5l/min.	0.02058	0.00172913	2.47669935
0.99	300	576	0.02307692	0.01904322	0.3306115	125 rpm	0.02058	0.00153678	2.59463194
0.4	360	144	1.10769231	0.00923308	0.64118594	30%w/v	0.0247	0.01546692	0.46809953
0.83	360	288	1.19076923	0.01915864	0.66523041	30°C	0.0247	0.00554136	1.49456253
0.99	360	432	0.44307692	0.02285187	0.5289784	1.5l/min.	0.0247	0.00184813	2.5926272
1	360	576	0.02769231	0.02308269	0.40074121	125 rpm	0.0247	0.00161731	2.72604129
0.4	420	144	1.29230769	0.01077192	0.74805026	35%w/v	0.02881	0.01803808	0.46823768
0.88	420	288	1.55076923	0.02369823	0.82285529	30°C	0.02881	0.00511177	1.72917727
0.99	420	432	0.35538462	0.02666051	0.61714146	1.5l/min.	0.02881	0.00214949	2.59549254
1	420	576	0.03230769	0.02692981	0.46753141	125 rpm	0.02881	0.00188019	2.72934938
0.55	180	144	0.76153846	0.00634774	0.44081533	15%w/v	0.01316	0.00681226	0.65845811
0.99	180	288	0.60923077	0.01142593	0.3967338	35°C	0.01316	0.00173407	2.02671264
1.03	180	432	0.05538462	0.01188759	0.27517563	1.5l/min.	0.01316	0.00127241	2.33626705
1.12	180	576	0.12461538	0.01292631	0.22441508	125 rpm	0.01316	0.00023369	4.03093534
0.43	240	144	0.79384615	0.00661704	0.45951659	20%w/v	0.01754	0.01092296	0.47361689
0.96	240	288	0.97846154	0.01477292	0.51294875	35°C	0.01754	0.00276708	1.84669282

1.03	240	432	0.12923077	0.01585012	0.36690084	1.5l/min.	0.01754	0.00168988	2.33982432
1.09	240	576	0.11076923	0.01677342	0.29120528	125 rpm	0.01754	0.00076658	3.13030558
0.7	300	144	1.61538462	0.0134649	0.93506283	25%w/v	0.02193	0.0084651	0.95190429
1.03	300	288	0.76153846	0.01981265	0.68793908	35°C	0.02193	0.00211735	2.33768812
1.07	300	432	0.09230769	0.02058207	0.47643677	1.5l/min.	0.02193	0.00134793	2.78928444
1.09	300	576	0.04615385	0.02096678	0.3640066	125 rpm	0.02193	0.00096322	3.12532917
0.5	360	144	1.38461538	0.01154135	0.80148242	30%w/v	0.02631	0.01476865	0.57744219
0.99	360	288	1.35692308	0.02285187	0.7934676	35°C	0.02631	0.00345813	2.02922019
1.09	360	432	0.27692308	0.02516014	0.58241056	1.5l/min.	0.02631	0.00114986	3.13030558
1.09	360	576	0	0.02516014	0.43680792	125 rpm	0.02631	0.00114986	3.13030558
0.42	420	144	1.35692308	0.01131052	0.78545277	35%w/v	0.0307	0.01938948	0.459532
0.69	420	288	0.87230769	0.01858157	0.64519335	35°C	0.0307	0.01211843	0.92953509
1.07	420	432	1.22769231	0.0288149	0.66701148	1.5l/min.	0.0307	0.0018851	2.79027968
1.07	420	576	0	0.0288149	0.50025861	125 rpm	0.0307	0.0018851	2.79027968

APPENDIX O

CALCULATION OF PYRITIC SULPHUR REMOVAL (BASED ON KINETIC EQUATION)

Ea	R	T	k= A.e(-E/RT)	k.t576 (lnC_t/Co)	k.t432 (lnC_t/Co)	k.t288 (lnC_t/Co)	k.t144 (lnC_t/Co)
10629.7274	1.986	303	0.00269789	1.553985429	1.165489071	0.776992714	0.388496357
10629.7274	1.986	308	0.00359388	2.070072262	1.552554197	1.035036131	0.517518066
10629.7274	1.986	313	0.00474376	2.732405823	2.049304368	1.366202912	0.683101456
10629.7274	1.986	318	0.00620714	3.575310077	2.681482558	1.787655038	0.893827519
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.01339	4.730284878	3.207491192	2.17492181	1.474761611
126648.485			0.01304	7.925395806	4.723519587	2.815207951	1.677858144
126648.485			0.01385	15.36981965	7.762499382	3.920436155	1.98000913
126648.485			0.01443	35.70569061	14.60673258	5.975423885	2.444468017
126648.485				Ct	Ct	Ct	Ct
				0.002830696	0.004174602	0.006156543	0.009079434
Condition				0.001645344	0.002760653	0.004631985	0.007771813
15%w/v				0.000901117	0.001784219	0.00353277	0.006994917
1.0 l/min.				0.000404137	0.000987901	0.002414891	0.005903125
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.010559304	0.009215398	0.007233457	0.004310566
				0.011394656	0.010279347	0.008408015	0.005268187
				0.012948883	0.012065781	0.01031723	0.006855083
				0.014025863	0.013442099	0.012015109	0.008526875
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				1266.060511	1104.926188	867.2914746	516.8369214
				1366.219291	1232.493668	1008.121041	631.6556023
				1552.571117	1446.687125	1237.035854	821.9244229
				1681.700942	1611.707717	1440.611516	1022.372325
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
8930.049	1.986	303	0.00252115	1.452181964	1.089136473	0.726090982	0.363045491
8930.049	1.986	308	0.00320792	1.84776213	1.385821597	0.923881065	0.461940532
8930.049	1.986	313	0.00405047	2.333073565	1.749805174	1.166536782	0.583268391
8930.049	1.986	318	0.00507696	2.924326256	2.193244692	1.462163128	0.731081564
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.01785	4.272426632	2.971706814	2.066984913	1.43770126
7022.63344			0.01739	6.345602985	3.998109392	2.519048031	1.587150916
7022.63344			0.01847	10.30958005	5.753481636	3.210853477	1.791885453
7022.63344			0.01924	18.62167558	8.964252216	4.315283952	2.077326154
7022.63344				Ct	Ct	Ct	Ct
				0.004177954	0.006006649	0.008635767	0.012415653
Condition				0.00274048	0.004349556	0.006903402	0.01095674
20%w/v				0.001791538	0.00321023	0.005752365	0.010307579
1.0 l/min.				0.001033205	0.002146303	0.004458571	0.009261906

125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.013672046	0.011843351	0.009214233	0.005434347
				0.01464952	0.013040444	0.010486598	0.00643326
				0.016678462	0.01525977	0.012717635	0.008162421
				0.018206795	0.017093697	0.014781429	0.009978094
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				1639.27837	1420.017775	1104.786543	651.5782022
				1756.477411	1563.549256	1257.343158	771.3478689
				1999.747645	1829.64641	1524.84448	978.6743358
				2182.994774	2049.534289	1772.293334	1196.373446
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
8846.00148	1.986	303	0.00339591	1.956045417	1.467034063	0.978022708	0.489011354
8846.00148	1.986	308	0.00431119	2.483243181	1.862432386	1.241621591	0.620810795
8846.00148	1.986	313	0.00543158	3.128588011	2.346441008	1.564294006	0.782147003
8846.00148	1.986	318	0.0067936	3.913113979	2.934835484	1.956556989	0.978278495
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.02231	7.071307624	4.336354692	2.65919304	1.630703235
8226.21394			0.02174	11.98005497	6.439380798	3.461221601	1.860435863
8226.21394			0.02308	22.84170453	10.448318	4.779299586	2.186160924
8226.21394			0.02404	50.05457843	18.81840695	7.074926037	2.659873312
8226.21394				Ct	Ct	Ct	Ct
				0.003155003	0.005144874	0.008389763	0.013681214
Condition				0.001814683	0.003376101	0.006281019	0.011685434
25%w/v				0.001010432	0.002208968	0.00482916	0.01055732
1.0 l/min.				0.000480276	0.001277473	0.003397915	0.009038024
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.019154997	0.017165126	0.013920237	0.008628786
				0.019925317	0.018363899	0.015458981	0.010054566
				0.022069568	0.020871032	0.01825084	0.01252268
				0.023559724	0.022762527	0.020642085	0.015001976
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				2296.684086	2058.098566	1669.036388	1034.591436
				2389.045529	2201.831489	1853.531775	1205.542495
				2646.141146	2502.436738	2188.275776	1501.469357
				2824.810938	2729.227031	2474.985946	1798.73687
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
9215.5365	1.986	303	0.00374938	2.159643112	1.619732334	1.079821556	0.539910778
9215.5365	1.986	308	0.00480761	2.769183924	2.076887943	1.384591962	0.692295981
9215.5365	1.986	313	0.00611575	3.52267113	2.642003348	1.761335565	0.880667783
9215.5365	1.986	318	0.00772117	4.447393907	3.33554543	2.223696954	1.111848477
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.02678	8.668043588	5.051737958	2.944154138	1.715853764
16784.175			0.02608	15.94561587	7.979597274	3.993196197	1.998298325
16784.175			0.0277	33.87479171	14.04130507	5.820205469	2.412510201
16784.175			0.02885	85.40408277	28.09370221	9.241432939	3.039972523
16784.175				Ct	Ct	Ct	Ct

				0.003089509	0.005301146	0.009095991	0.015607391
Condition				0.001635559	0.003268335	0.006531109	0.013051104
30%w/v				0.000817717	0.001972751	0.004759282	0.011481817
1.0 l/min.				0.000337806	0.001026921	0.00312181	0.009490217
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.023690491	0.021478854	0.017684009	0.011172609
				0.024444441	0.022811665	0.019548891	0.013028896
				0.026882283	0.025727249	0.022940718	0.016218183
				0.028512194	0.027823079	0.02572819	0.019359783
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				2840.489853	2575.314606	2120.31266	1339.59586
				2930.888442	2735.118589	2343.91202	1562.164586
				3223.185707	3084.697143	2750.59207	1944.560173
				3418.612077	3335.987227	3084.809952	2321.237939
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
14237.3758	1.986	303	0.00222869	1.283727132	0.962795349	0.641863566	0.320931783
14237.3758	1.986	308	0.00327232	1.884858372	1.413643779	0.942429186	0.471214593
14237.3758	1.986	313	0.00474606	2.733729003	2.050296752	1.366864501	0.683432251
14237.3758	1.986	318	0.00680349	3.91881095	2.939108212	1.959405475	0.979702737
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.03124	3.610069889	2.61900729	1.900018392	1.378411547
42004904.3			0.03043	6.585421695	4.110907384	2.566207649	1.601938716
42004904.3			0.03232	15.39017013	7.770206588	3.923030733	1.980664215
42004904.3			0.03366	50.34055173	18.89898491	7.095107591	2.663664317
42004904.3				Ct	Ct	Ct	Ct
				0.008653572	0.011928184	0.016441946	0.022663768
Condition				0.004620813	0.007402259	0.011857965	0.018995733
35%w/v				0.002100042	0.004159478	0.008238528	0.016317758
1.0 l/min.				0.000668646	0.001781048	0.004744114	0.012636727
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.022586428	0.019311816	0.014798054	0.008576232
				0.025809187	0.023027741	0.018572035	0.011434267
				0.030219958	0.028160522	0.024081472	0.016002242
				0.032991354	0.031878952	0.028915886	0.021023273
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				2708.112708	2315.486777	1774.286662	1028.290173
				3094.521561	2761.02618	2226.787019	1370.968623
				3623.372993	3376.44664	2887.368448	1918.668776
				3955.663365	3822.28634	3467.014719	2520.690378
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
5307.7836	1.986	303	0.00413735	2.383112191	1.787334143	1.191556095	0.595778048
5307.7836	1.986	308	0.00477428	2.749988131	2.062491098	1.374994065	0.687497033
5307.7836	1.986	313	0.00548413	3.158859914	2.369144936	1.579429957	0.789714979
5307.7836	1.986	318	0.00627212	3.612741155	2.709555867	1.806370578	0.903185289
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.01235	10.83858216	5.973506706	3.292200201	1.814442118

28.0156046			0.01316	15.64244622	7.865539254	3.955053251	1.988731568
28.0156046			0.01489	23.54373874	10.68824923	4.852189066	2.2027685
28.0156046			0.01466	37.06752152	15.02260199	6.088310235	2.467450148
28.0156046				Ct	Ct	Ct	Ct
				0.001139448	0.002067462	0.003751291	0.0068065
Condition				0.000841301	0.001673121	0.003327389	0.006617283
15%w/v				0.00063244	0.001393119	0.003068718	0.006759675
1.5 l/min.				0.000395494	0.000975863	0.002407893	0.005941356
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.011210552	0.010282538	0.008598709	0.0055435
				0.012318699	0.011486879	0.009832611	0.006542717
				0.01425756	0.013496881	0.011821282	0.008130325
				0.014264506	0.013684137	0.012252107	0.008718644
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				1344.145193	1232.876269	1030.985245	664.6656684
				1477.012053	1377.276775	1178.93008	784.4717441
				1709.481455	1618.276066	1417.37171	974.8259219
				1710.314212	1640.728038	1469.027621	1045.365403
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
2277.8705	1.986	303	0.00392075	2.258352068	1.693764051	1.129176034	0.564588017
2277.8705	1.986	308	0.00416924	2.401481933	1.80111145	1.200740966	0.600370483
2277.8705	1.986	313	0.00442478	2.548674409	1.911505807	1.274337204	0.637168602
2277.8705	1.986	318	0.00468721	2.699833429	2.024875071	1.349916714	0.674958357
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.01647	9.567309891	5.439918346	3.093106835	1.758723069
0.17271498			0.01754	11.0395241	6.05637508	3.322577929	1.82279399
0.17271498			0.01985	12.79013805	6.763265304	3.576330248	1.891118782
0.17271498			0.01954	14.87725339	7.575164531	3.857104276	1.96395119
0.17271498				Ct	Ct	Ct	Ct
				0.001721487	0.003027619	0.005324743	0.009364749
Condition				0.001588837	0.002896122	0.005279033	0.00962259
20%w/v				0.001551977	0.002934973	0.005550382	0.010496432
1.5 l/min.				0.001313414	0.002579482	0.005065977	0.009949331
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.014748513	0.013442381	0.011145257	0.007105251
				0.015951163	0.014643878	0.012260967	0.00791741
				0.018298023	0.016915027	0.014299618	0.009353568
				0.018226586	0.016960518	0.014474023	0.009590669
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				1768.346704	1611.741485	1336.316274	851.9196021
				1912.544491	1755.800994	1470.08989	949.2974076
				2193.932958	2028.111756	1714.524166	1121.492784
				2185.367604	2033.566117	1735.435406	1149.921241
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
4470.77993	1.986	303	0.00503948	2.902739161	2.177054371	1.451369581	0.72568479
4470.77993	1.986	308	0.00568546	3.274823927	2.456117945	1.637411963	0.818705982

4470.77993	1.986	313	0.00638957	3.680394872	2.760296154	1.840197436	0.920098718
4470.77993	1.986	318	0.00715458	4.121035315	3.090776486	2.060517658	1.030258829
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.02058	18.22399553	8.820286663	4.268957195	2.066145492
8.49160922			0.02193	26.43856995	11.6594609	5.141844995	2.267563669
8.49160922			0.02481	39.66205242	15.80452283	6.297781548	2.509538114
8.49160922			0.02443	61.62300848	21.9941495	7.850032387	2.801790925
8.49160922				Ct	Ct	Ct	Ct
				0.00112928	0.002333257	0.004820849	0.009960576
Condition				0.00082947	0.001880876	0.004265006	0.009671173
25%w/v				0.000625535	0.001569804	0.003939482	0.009886281
1.5 l/min.				0.000396443	0.00111075	0.003112089	0.008719423
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.01945072	0.018246743	0.015759151	0.010619424
				0.02110053	0.020049124	0.017664994	0.012258827
				0.024184465	0.023240196	0.020870518	0.014923719
				0.024033557	0.02331925	0.021317911	0.015710577
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				2332.141286	2187.784426	1889.522149	1273.268891
				2529.95355	2403.88997	2118.032775	1469.833386
				2899.717359	2786.499524	2502.375052	1789.353859
				2881.623504	2795.978084	2556.01752	1883.698192
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
2949.42846	1.986	303	0.0053879	3.103429355	2.327572016	1.551714677	0.775857339
2949.42846	1.986	308	0.00583412	3.36045091	2.520338183	1.680225455	0.840112728
2949.42846	1.986	313	0.00630125	3.629520317	2.722140238	1.814760158	0.907380079
2949.42846	1.986	318	0.00678932	3.910650178	2.932987633	1.955325089	0.977662544
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.0247	22.27420661	10.25301713	4.719555764	2.172453858
0.72451937			0.02631	28.80217515	12.43280051	5.3667658	2.31662811
0.72451937			0.02978	37.69473075	15.21284653	6.139603468	2.477822324
0.72451937			0.02932	49.93140572	18.78366546	7.066215799	2.658235467
0.72451937				Ct	Ct	Ct	Ct
				0.001108906	0.002409047	0.005233543	0.011369632
Condition				0.000913473	0.002116176	0.004902394	0.011357024
30%w/v				0.000790031	0.001957556	0.004850476	0.012018618
1.5 l/min.				0.000587206	0.001560931	0.004149321	0.011029873
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.023591094	0.022290953	0.019466457	0.013330368
				0.025396527	0.024193824	0.021407606	0.014952976
				0.028989969	0.027822444	0.024929524	0.017761382
				0.028732794	0.027759069	0.025170679	0.018290127
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				2828.572178	2672.685266	2334.028144	1598.311173
				3045.043626	2900.83944	2566.77197	1792.861876
				3475.897302	3335.911028	2989.049913	2129.589701
				3445.062051	3328.31241	3017.964371	2192.98621

Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
2168.70008	1.986	303	0.00521697	3.004975828	2.253731871	1.502487914	0.751243957
2168.70008	1.986	308	0.0055313	3.186028169	2.389521127	1.593014085	0.796507042
2168.70008	1.986	313	0.00585361	3.371680875	2.528760656	1.685840437	0.842920219
2168.70008	1.986	318	0.00618368	3.561802445	2.671351834	1.780901223	0.890450611
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.02881	20.18572816	9.523208992	4.492853009	2.119635112
0.19168583			0.0307	24.19214925	10.90826902	4.91855154	2.217780769
0.19168583			0.03474	29.12744551	12.53795766	5.396984854	2.323141161
0.19168583			0.0342	35.22663403	14.45950283	5.935202948	2.436227195
0.19168583				Ct	Ct	Ct	Ct
				0.001427246	0.003025241	0.006412407	0.013591962
Condition				0.001269007	0.002814379	0.006241675	0.013842667
35%w/v				0.00119269	0.002770786	0.006436927	0.014953891
1.5 l/min.				0.000970856	0.002365227	0.005762229	0.0140381
125 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.027382754	0.025784759	0.022397593	0.015218038
				0.029430993	0.027885621	0.024458325	0.016857333
				0.03354731	0.031969214	0.028303073	0.019786109
				0.033229144	0.031834773	0.028437771	0.0201619
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				3283.192203	3091.592633	2685.471456	1824.642751
				3528.776094	3343.485994	2932.553172	2021.194263
				4022.322522	3833.108735	3393.538398	2372.354454
				3984.174328	3816.989337	3409.688714	2417.411859
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
3991.34364	1.986	303	0.00525252	3.025454323	2.269090742	1.512727162	0.756363581
3991.34364	1.986	308	0.00584966	3.369406716	2.527055037	1.684703358	0.842351679
3991.34364	1.986	313	0.00649232	3.739575048	2.804681286	1.869787524	0.934893762
3991.34364	1.986	318	0.00718199	4.136828549	3.102621412	2.068414275	1.034207137
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.01189	20.60336317	9.670603745	4.53909277	2.130514672
3.98987096			0.01339	29.06128034	12.51659091	5.390851541	2.321820738
3.98987096			0.01581	42.08010435	16.52180934	6.48691794	2.546942861
3.98987096			0.01408	62.60396088	22.25621759	7.912266482	2.812875127
3.98987096				Ct	Ct	Ct	Ct
				0.00057709	0.001229499	0.002619466	0.005580811
Condition				0.000460751	0.00106978	0.002483838	0.005767026
15%w/v				0.000375712	0.000956917	0.002437213	0.006207442
1.5 l/min.				0.000224906	0.000632632	0.001779515	0.005005555
175 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.01131291	0.010660501	0.009270534	0.006309189
				0.012929249	0.01232022	0.010906162	0.007622974
				0.015434288	0.014853083	0.013372787	0.009602558
				0.013855094	0.013447368	0.012300485	0.009074445
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)

				1356.417879	1278.194041	1111.537005	756.4717453
				1550.217013	1477.194365	1307.648866	913.9946118
				1850.571132	1780.884653	1603.397175	1151.346708
				1661.225783	1612.339399	1474.828104	1088.026005
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
2696.14395	1.986	303	0.00519347	2.99143592	2.24357694	1.49571796	0.74785898
2696.14395	1.986	308	0.00558529	3.217124726	2.412843544	1.608562363	0.804281181
2696.14395	1.986	313	0.00599273	3.451810023	2.588857517	1.725905012	0.862952506
2696.14395	1.986	318	0.00641567	3.695423949	2.771567962	1.847711975	0.923855987
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.01585	19.91425724	9.42699083	4.462539327	2.112472326
0.45844791			0.01785	24.95626083	11.16566611	4.995624169	2.235089298
0.45844791			0.02108	31.55746038	13.31455127	5.617602725	2.37014825
0.45844791			0.01877	40.26263829	15.98367617	6.345284729	2.518984861
0.45844791				Ct	Ct	Ct	Ct
				0.000795912	0.001681342	0.003551789	0.007503057
Condition				0.000715251	0.001598651	0.003573127	0.007986258
20%w/v				0.000667988	0.00158323	0.00375249	0.008893958
1.5 l/min.				0.000466189	0.001174323	0.002958102	0.007451414
175 rpm				∑Pymv= Co-Ct	∑Pymv= Co-Ct	∑Pymv= Co-Ct	∑Pymv= Co-Ct
				0.015054088	0.014168658	0.012298211	0.008346943
				0.017134749	0.016251349	0.014276873	0.009863742
				0.020412012	0.01949677	0.01732751	0.012186042
				0.018303811	0.017595677	0.015811898	0.011318586
				∑Pymv(mg/l)	∑Pymv(mg/l)	∑Pymv(mg/l)	∑Pymv(mg/l)
				1804.985129	1698.822038	1474.555448	1000.798481
				2054.45636	1948.536781	1711.797063	1182.662654
				2447.400257	2337.662696	2077.568407	1461.106385
				2194.626936	2109.721661	1895.84656	1357.098417
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo/Ct)	k.t288 (lnCo/Ct)	k.t144 (lnCo/Ct)
4259.56486	1.986	303	0.00444786	2.561969414	1.921477061	1.280984707	0.640492354
4259.56486	1.986	308	0.0049895	2.873950437	2.155462828	1.436975218	0.718487609
4259.56486	1.986	313	0.00557658	3.212108362	2.409081272	1.606054181	0.803027091
4259.56486	1.986	318	0.00621097	3.577518521	2.683138891	1.78875926	0.89437963
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.01981	12.9613184	6.831040883	3.600183107	1.897414848
5.27618482			0.02231	17.70682993	8.631884328	4.207948422	2.051328453
5.27618482			0.02635	24.83138463	11.12373676	4.983109936	2.232288049
5.27618482			0.02347	35.78463177	14.63094624	5.982025724	2.445818007
5.27618482				Ct	Ct	Ct	Ct
				0.001528394	0.002899997	0.005502498	0.010440521
Condition				0.001259966	0.002584604	0.005301871	0.010875879
25%w/v				0.001061157	0.002368808	0.005287862	0.011804032
1.5 l/min.				0.000655868	0.001604134	0.00392342	0.009595972
175 rpm				∑Pymv= Co-Ct	∑Pymv= Co-Ct	∑Pymv= Co-Ct	∑Pymv= Co-Ct
				0.018281606	0.016910003	0.014307502	0.009369479

				0.021050034	0.019725396	0.017008129	0.011434121
				0.025288843	0.023981192	0.021062138	0.014545968
				0.022814132	0.021865866	0.01954658	0.013874028
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				2191.964571	2027.509326	1715.469501	1123.400505
				2523.899101	2365.075019	2039.274659	1370.951111
				3032.132264	2875.344884	2525.35029	1744.06154
				2735.41441	2621.71732	2343.634929	1663.496012
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo/Ct)	k.t432 (lnCo-Ct)	k.t288 (lnCo-Ct)	k.t144 (lnCo-Ct)
2857.79839	1.986	303	0.00512837	2.953943299	2.215457474	1.476971649	0.738485825
2857.79839	1.986	308	0.00553939	3.190687735	2.393015801	1.595343867	0.797671934
2857.79839	1.986	313	0.00596862	3.437927658	2.578445743	1.718963829	0.859481914
2857.79839	1.986	318	0.00641605	3.695642101	2.771731576	1.84782105	0.923910525
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.02378	19.18144295	9.165601177	4.379662424	2.092764302
0.59221581			0.02678	24.30513718	10.94645655	4.930024055	2.220365748
0.59221581			0.0316	31.12239503	13.17664235	5.578744933	2.361936691
0.59221581			0.02816	40.2714226	15.98629153	6.345976883	2.519122245
0.59221581				Ct	Ct	Ct	Ct
				0.00123974	0.002594483	0.005429642	0.011362961
Condition				0.001101825	0.002446454	0.005432022	0.012061076
30%w/v				0.001015346	0.002398183	0.005664356	0.013378851
1.5 l/min.				0.000699255	0.001761509	0.004437457	0.011178497
175 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.02254026	0.021185517	0.018350358	0.012417039
				0.025678175	0.024333546	0.021347978	0.014718924
				0.030584654	0.029201817	0.025935644	0.018221149
				0.027460745	0.026398491	0.023722543	0.016981503
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				2702.577187	2540.143442	2200.207898	1488.802927
				3078.813221	2917.592192	2559.622541	1764.798989
				3667.100012	3501.297855	3109.683658	2184.715718
				3292.543306	3165.179044	2844.332897	2036.082231
Ea	R	T	k= A.e(-E/RT)	k.t576 (lnCo-Ct)	k.t432 (lnCo-Ct)	k.t288 (lnCo-Ct)	k.t144 (lnCo-Ct)
4896.6816	1.986	303	0.00492287	2.835572261	2.126679196	1.417786131	0.708893065
4896.6816	1.986	308	0.00561808	3.236015201	2.427011401	1.618007601	0.8090038
4896.6816	1.986	313	0.00638447	3.677455997	2.758091998	1.838727999	0.919363999
4896.6816	1.986	318	0.00722629	4.162344022	3.121758017	2.081172011	1.040586006
			Co	Co/Ct	Co/Ct	Co/Ct	Co/Ct
A			0.02774	17.04014893	8.386969034	4.127971527	2.031741009
16.8344914			0.03124	25.43217746	11.32498561	5.043032566	2.245669737
16.8344914			0.03689	39.54566173	15.76972556	6.288534148	2.507694987
16.8344914			0.03285	64.22188383	22.68622737	8.013855741	2.830875437
16.8344914				Ct	Ct	Ct	Ct
				0.00162792	0.003307512	0.006720008	0.013653315
Condition				0.001228365	0.002758502	0.006194685	0.013911217
35%w/v				0.000932846	0.002339292	0.005866232	0.01471072

1.5 l/min.				0.000511508	0.001448015	0.00409915	0.011604184
175 rpm				ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct	ΣPymv= Co-Ct
				0.02611208	0.024432488	0.021019992	0.014086685
				0.030011635	0.028481498	0.025045315	0.017328783
				0.035957154	0.034550708	0.031023768	0.02217928
				0.032338492	0.031401985	0.02875085	0.021245816
				ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)	ΣPymv(mg/l)
				3130.838387	2929.455322	2520.29709	1688.993531
				3598.395021	3414.931562	3002.933225	2077.721029
				4311.262802	4142.629835	3719.749788	2659.295615
				3877.385205	3765.097988	3447.226867	2547.373315

APPENDIX P

ORGANIC SULPHUR CONTENT BEFORE AND AFTER TREATMENT

Experimental conditions	%w/v	% organic sulphur	
		before	after
1. 30°C; 1.5 l/min.;125 rpm	15	1.53	1.51
	20	1.53	1.53
	25	1.53	1.53
	30	1.53	1.50
	35	1.53	1.50
2. 40°C; 1.5 l/min.;125 rpm	15	1.31	1.31
	20	1.31	1.30
	25	1.31	1.30
	30	1.31	1.29
	35	1.31	1.29
3. 45°C; 1.5 l/min.;125 rpm	15	1.31	1.31
	20	1.31	1.27
	25	1.31	1.29
	30	1.31	1.28
	35	1.31	1.29
4. 30°C; 1.5 l/min.;175 rpm	15	1.54	1.53
	20	1.54	1.54
	25	1.54	1.53
	30	1.54	1.52
	35	1.54	1.54
5. 35°C; 1.5 l/min.;175 rpm	15	1.44	1.44
	20	1.44	1.41
	25	1.44	1.44
	30	1.44	1.39
	35	1.44	1.40
4. 40°C; 1.5 l/min.;175 rpm	15	1.27	1.26
	20	1.27	1.27
	25	1.27	1.28
	30	1.27	1.26
	35	1.27	1.27

APPENDIX Q

PUBLISHED PAPERS

(1). "**Coal Desulphurisation in Stirred Batch Reactor Using *Thiobacillus ferrooxidans* Dominant, Mixed Bacterial Cultures**". In "Proceedings of Chemeca 90, The Eighteenth Australasian Chemical Engineering Conference, Processing Pasific Resources", volume II, August 27-30, 1990, Auckland, New Zealand, p. 1109-1115.

(2). "**The Correlation of Pyritic Sulphur Removal With pH Values From Coal In Water Slurries Containing Mixed Acidophilic Culture**". In "Proceedings of The Fourth New Zealand Coal Conference", October 14-16, 1991, Wellington, New Zealand, p. 49-57.

COAL DESULPHURISATION IN STIRRED BATCH REACTOR USING THIOBACILLUS FERROOXIDANS DOMINANT, MIXED BACTERIAL CULTURES

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ABSTRACT:

Coal desulphurisation experiments using acidophilic microbes, containing predominantly Thiobacillus ferrooxidans, were performed in 2 litre stirred batch reactor, under aerobic conditions.

The physical parameters which had greatest influence on the microbial process were temperature, slurry concentration, volume of air flow. The preliminary results show that different temperatures (ranging from 35° to 45°C) of the process yield different rates of pyritic sulphur oxidation, as indicated by pH changes during the experiment. The optimal condition for coal desuphurisation using this type of reactor is not yet final since the experiments are still in progress. However early trends show that the sulphur oxidizing activity of this particular culture is enhanced at temperatures close to 40°C.

INTRODUCTION

Almost all coals contain sulphur which occurs in two dominant forms, namely, inorganic, mainly pyritic sulphur and organic sulphur which may be chemically bound to the coal macromolecules or in the mobile phase. During the combustion of coal these sulphur compounds are mainly converted to sulphur oxides which have adverse effects on the biosphere and contribute to the acid rain problem. The removal of sulphur compounds, either before or after combustion, would significantly reduce the pollution of the environment. Various physical and chemical methods have been developed for desulphurisation of coal prior to combustion. Desulphurisation of coal by microbes offers significant operating and environmental advantages over the physical/ mechanical and chemical methods (Kargi and Robinson, 1985). One disadvantage of microbial coal desulphurisation is that it generally takes longer, requiring large reactor volumes and holding ponds on a commercial scale.

The majority of microbial coal desulphurisation experiments have been carried out using shaken flasks and detailed information on the rate controlling step in slurry bioreactors is scarce. This paper summarizes the preliminary results of a study in coal desulphurisation using the acidophilic mixed bacterial cultures dominated by Thiobacillus ferrooxidans in experimentally stirred batch reactors. This study examined the rate and extent of removal of pyritic sulphur from coal by optimizing the process parameters which affect pyrite oxidation rates, such as temperature, slurry concentration, and volume of air flow. By using stirred batch reactors these parameters could be varied quite easily and effectively controlled in order to observe their effects on the rates of microbial sulphur removal. This study demonstrates the diverse capabilities of Thiobacillus ferrooxidans dominant mixed bacterial cultures in oxidizing pyritic sulphur, at different temperatures, ranging from 35° to 45°C and different slurry concentrations (15% to 35% w/v).

CHEMICAL REACTIONS IN A MICROBIAL COAL DESULPHURISATION

The pioneering work of Colmer and Hinkle (1947) has prompted numerous studies on the process with proposed mechanisms of biodegradation of iron pyrites which have provided better insight into the biological breakdown processes. The mechanisms of these bioprocesses are either the direct attack of the pyritic sulphur by the bacteria or indirect dissolution of the pyritic sulphur via a bacterially generated oxidant (Brierly, 1978). Direct oxidation occurs via contact between the bacteria and the pyritic moiety of the coal. Pyritic sulphur is oxidized to sulfate and ferrous iron to ferric. The indirect mechanism can occur without bacteria intervention. Ferric iron which is produced by the bacteria's oxidative attack on the ferrous iron in pyrite, chemically oxidizes the ferrous moiety of pyrite. The major role of bacteria in the indirect mechanism is to regenerate the ferric iron and thus greatly accelerate the chemical oxidation of pyrite. Since the bacteria are strict aerobes, oxygen is required regardless of whether the reactions occur via the indirect or direct mechanism. In the case of iron pyrites, the overall oxidation, including direct and indirect oxidation, is described by equation 1.

$$4FeS_2 + 15O_2 + 2H_2O \text{ ----> } 2Fe_2(SO_4)_3 + 2H_2SO_4 \quad (1)$$

This overall oxidation reaction represents the sum of a number of reactions which are occuring to yield the two main products. The initiating reaction where pyrite is exposed to oxygen and water, is a slow spontaneous reaction.

It can be catalyzed by some bacteria, in which case the bacteria has an important role (equation 2):

$$2FeS_2 + 7O_2 + 2H_2O \text{ --(bacteria)----> } 2FeSO_4 + 2H_2SO_4 \quad (2)$$

The generation of hydrogen ions in this reaction leads to the development of acidic conditions, under which conditions the auto-oxidation of ferrous iron is very slow. In the presence of various bacteria, the best known of which is Thiobacillus ferrooxidans, the ferrous iron generated in this reaction can be rapidly oxidized by the oxygen. More specifically, Murr L.E., (1980), said this reaction is about 10 million times faster than the same reaction in the absence of bacteria.

$$4\ FeSO_4 + O_2 + 2H_2SO_4 \text{ --(bacteria)---> } 2\ Fe_2(SO_4)_3 + 2H_2O \quad (3)$$

This reaction (equation 3) is the rate limiting step in the overall pyrite oxidation and acidity forming process. The ferric ions produced from this reaction (equation 3) can chemically degrade pyrite, by the following reaction:

$$FeS_2 + Fe_2(SO_4)_3 + 3\ O_2 + 2H_2O \text{ ----> } 3FeSO_4 + 2H_2SO_4 \quad (4)$$

There is some evidence, that this reaction (equation 4) proceeds by two steps, one of which is bacterially catalyzed.

$$FeS_2 + Fe_2(SO_4)_3 \text{ -----> } 3\ FeSO_4 + 2S \quad (5)$$

$$2\ S + 3O_2 + 2\ H_2O \text{ --(bacteria)---> } 2H_2SO_4 \quad (6)$$

Reaction 6 is important because it is believed to prevent a layer of elemental sulphur from accumulating on pyrite surfaces, inhibiting further reactions (Tributch and Bennett, 1981). Some of the ferric ion produced may react to yield insoluble hydroxides (ferric hydroxide) and more acid (sulfate) according to the reaction:

$$Fe_2(SO_4)_3 + 6\ H_2O \text{ ------> } 2\ Fe(OH)_3 + 3H_2SO_4 \quad (7)$$

EXPERIMENTAL

Microbiological methods: The genus Thiobacillus is the best known group in the category of sulphur compound metabolizing bacteria. Thiobacillus ferrooxidans is a mesophilic (20°-45°C), gram negative, short-rod like, acidophilic bacterium. It is aerobic, obtains carbon in the form of CO_2 from air and derives its metabolic energy from the oxidation of inorganic substrates such as ferrous iron and reduced sulphur compounds as well as insoluble pyrite.

A strain of Thiobacillus ferrooxidans isolated from acid mine water at Stockton Plateau, New Zealand was used throughout these experiments. The composition of the mineral salts medium used for growing the culture was (in gram per litre): $(NH_4)_2SO_4$: 0.8; KH_2PO_4: 0.4; $MgSO_4$ $7H_2O$: 0.16 and $FeSO_4$ $7H_2O$: 20 gram. This is the same medium used in maintaining pure cultures of Thiobacillus ferrooxidans. The mineral salts were dissolved in distilled water and initially the pH of the medium was adjusted with 1M H_2SO_4 to the desired pH. The organisms were cultivated in shaken flasks in an incubator at a temperature of 30°C. Serial dilutions were made from the first culture produced. In order to adapt the organisms to pyrite, the cells grown on the growth medium were transferred to a salt medium containing 10% w/v coal particles (ground and screened to under 100 BSS mesh /152 microns) in place of $FeSO_4.7H_2O$. By a dilution enrichment technique and after three transfers in coal containing salt medium, cells were assumed to be adapted to pyrite in coal. The adapted cultures were used as the inoculum for the first experiments of coal desulphurisation in the stirred batch reactors. Each batch of the next experiments was inoculated with culture from the preceeding batch.

Coal desulphurisation experiments: Benneydale coal collected by Coal Research Association of New Zealand Inc. was used in this study. All coal samples were crushed and ground to particle size under 100 BSS mesh (152 microns). The composition of Benneydal coal was: Total moisture: 22.0%.Ash: 7.6%.Volatiles: 36.6%.Fixed-Carbon: 37.9%.Calerific-value: 22.37 (MJ/kg). Total Sulphur: 2.32%. Sulphate Sulphur: 0.03%. Pyritic Sulphur: 1.24%. Organic Sulphur: 1.05 % (On air dried basis at 70 % R.H).

Reactor and experimental conditions: The coal desulphurisation experiments were performed in six of 2 litres stirred batch reactors each with a working volume of 1.3 litres. The reactors were charged with 1200 ml mineral salts medium without $FeSO_4.7H_2O$ and coal particles of known particle size with slurry concentrations in the range of 15% to 35% w/v. The reactors were inoculated with 100 ml adapted cell liquid of Thiobacillus ferrooxidans. The initial pH of the inoculated medium was adjusted to pH 2.6 with 1M H_2SO_4

Experimental conditions: operating temperature: 35°, 40° and 45°C; Stirrer rotation: 125 rpm; Air flow rate: 1 litre per minute; CO_2 flow rate: 5 cc per minute. Each reactor was equipped with separate air and CO_2 lines. In order to reduce the evaporation rate of the liquid, the gases were saturated by bubbling through water and evaporation losses were compensated for by topping up with distilled water every day. The experiment ran for 24 days. A schematic diagram of the experimental set up was shown in figure 1.

Analytical methods: The pH measurements were conducted every day to monitor the rate of oxidation. Redox potential of the liquid was measured at the start and the end of the process.

1. pH measurements of the liquid-solid mixture in the reactors were taken using a pH meter (Radiometer, type PHM-26c, Copenhagen, Sweden).
2. A portable transistorised pH meter (Metrohom-Herisau, model E 488, Switzerland) was used to measure redox potential of the mixture in the reactors.
3. Coal samples were withdrawn from the reactors every 6 days and filtered through Whatman no. 41 filter paper and analyzed for sulphur content.

For chemical analyses the coal samples were prepared and examined as followed: Total Sulphur: Standard ASTM D 4239, a high temperature tube furnace combustion method. Forms of sulphur (pyritic, sulphate): AS 1038, part 11, 1982. The organic sulphur content of coal samples was determined indirectly from the difference between total sulphur and total inorganic sulphur content of coal. Sulphur determinations were carried out by Coal Research Association, Gracefield, New Zealand.

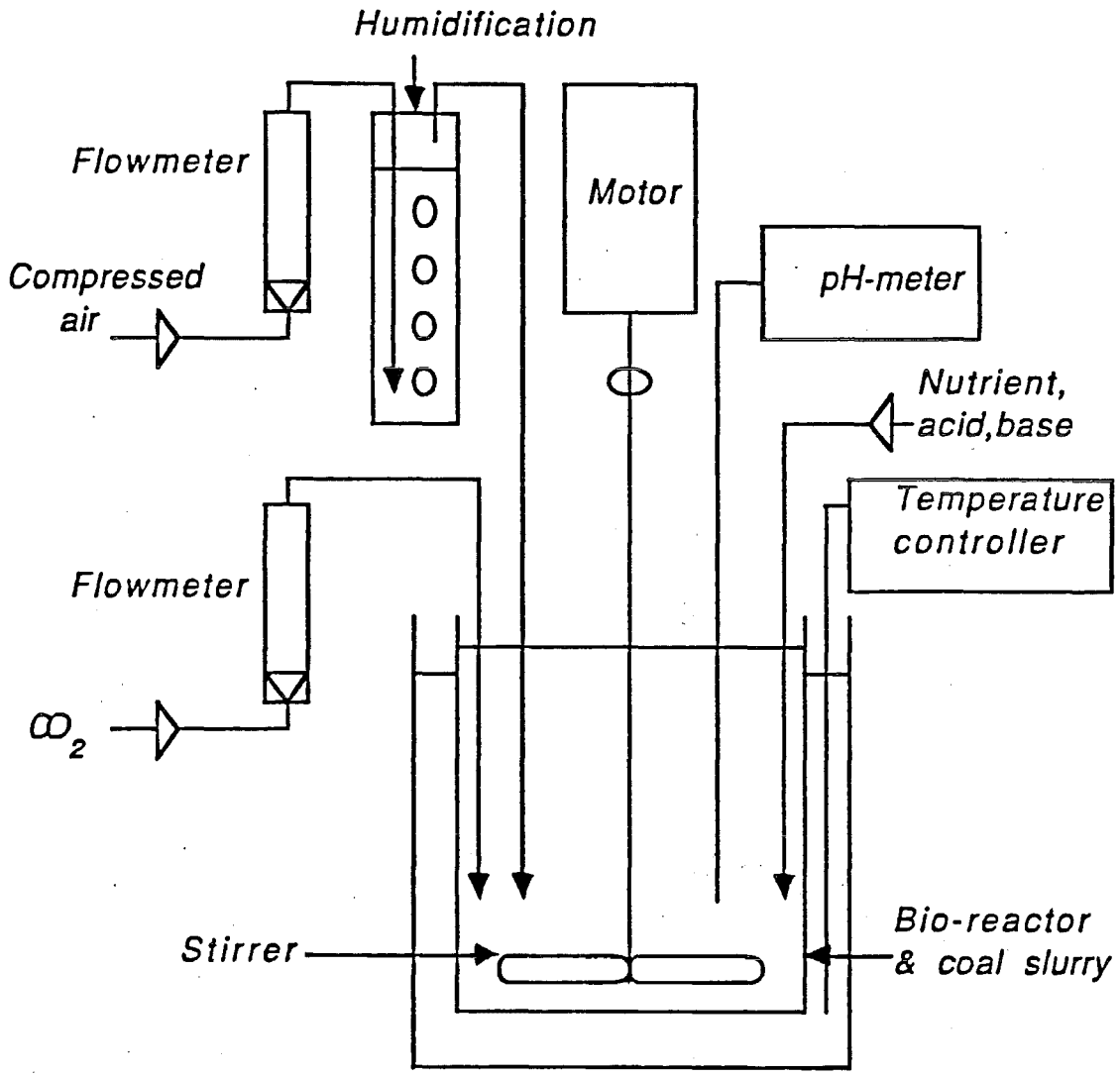

Figure 1: Schematic diagram of the experimental setup

RESULTS AND DISCUSSION

Effect of different slurry concentrations: Investigation of the performance of microbial coal desulphurisation at high slurry concentrations is important especially from industrial and economical point of view. Data from these experiments at different temperatures (35°, 40° and 45°C) shows that the increase of slurry concentration leads to an increase in the absolute amount of soluble product (sulphuric acid) released in the liquid. See tables I, II and III. The sulphuric acid data shows the development of desulphurisation, although it is difficult to correlate this with the actual amount of iron pyrite which has been converted. It must be noted that the amount of sulphuric acid produced generally relates to the amount of iron pyrite available to be converted by the microorganisms during the experiment. However, this product does not always give an indication of the actual amount of iron pyrite removed from coal. This result was confirmed by other investigators (Hone, et al, 1987). More experimental work is needed in order to elucidate the relationship between the sulphuric acid production and the iron pyrite reduction.

Effect of temperature: To examine the effect of different temperatures on the rate of coal desulphurisation, a quantitative treatment can be given by van't Hoff temperature coefficient (Trumbore, 1966 and Voznaya, 1981). The mathematical expression on the dependence of reaction rate on temperature is:

$$Q=(k_2/k_1)^{10/t_2-t_1}$$

where k_1 is the initial reaction rate of temperature t_1; k_2 is the reaction rate after a rise in temperature to t_2; Q is temperature coefficient. Increase in temperature leads to the increase of sulphuric acid produced and its development can be shown by the profile of temperature coefficient in figures 2 and 3. Figure 2 shows the profile of temperature coefficient between 35° and 40°C. After 12 days the reaction rate was greater as indicated by a value between 1 and 2. It further increased until the run was stopped. Figure 3 shows different rate trends. An increase in temperature from 40° to 45°C shows a clear maximum value after the first 6 days. It can be interpreted that most of the iron pyrite oxidation took place during that period. Most of the reactions were enhanced, reaching maximum value and at the same time, concentration of iron pyrite decreased. This trend was also confirmed by the results for percent pyrite reduction and sulphuric acid concentrations in table III.

Table I: Sulphuric Acid (mg/l) and Pyrite Reduction (%) at 35° C, 125 rpm

Days	Sulphuric Acid					Pyrite Reduction				
	15%	20%	25%	30%	35%	15%	20%	25%	30%	35%
6	209	295	458	427	364	28.0	45.8	54.2	54.9	45.8
12	538	590	742	814	590	82.2	76.6	87.9	86.0	52.3
18	725	777	1025	978	814	95.3	92.5	93.5	91.6	72.0
24	934	1025	1320	1176	1025	95.3	93.5	98.5	94.4	83.2

Table II: Sulphuric Acid (mg/l) and Pyrite Reduction (%) at 40°C, 125 rpm

Days	Sulphuric Acid					Pyrite Reduction				
	15%	20%	25%	30%	35%	15%	20%	25%	30%	35%
6	204	209	252	235	209	54.2	56.7	30.8	42.5	44.2
12	646	814	852	814	892	81.7	80.0	70.8	76.7	66.7
18	934	1302	1624	1822	1624	85.0	83.3	90.0	92.5	88.3
24	1414	1700	2141	2515	2294	94.1	90.8	97.5	95.8	95.0

Pyrite reduction: These experiments showed the reduction of iron pyrite around (83.2% - 98.4%). Almost all of the iron pyrite has been removed after 12 days. The rate was initially low due to low microbial activity. During the first 6 days the reduction of iron pyrite was about (28.0% - 63.2%) depending on temperature and slurry concentration. It increased, thereafter reaching the maximum value mostly between 12 and 18 days. After 18 days, there was only slight reduction and at the end of the experimental period most of the iron pyrite has been converted. These results suggest that the overall oxidation rate is not exclusively controlled by microbial activity but also by the amount of iron pyrite available.

Table III: Sulphuric Acid (mg/l) and Pyrite Reduction (%) at 45°C, 125 rpm

Days	Sulphuric Acid					Pyrite Reduction				
	15%	20%	25%	30%	35%	15%	20%	25%	30%	35%
6	347	246	301	563	458	63.2	50.4	32.8	46.4	38.4
12	1025	513	1320	1624	1447	90.4	63.2	83.2	91.2	60.8
18	1073	590	1587	1780	1822	93.6	83.2	90.4	95.2	85.6
24	1261	646	1700	1952	2141	93.6	85.6	96.0	98.4	97.6

CONCLUSION

The Thiobacillus ferrooxidans dominant mixed culture was effective in removing iron pyrite from coal. Most of the iron pyrite can be removed after 12 days of microbial activity. An increase in slurry concentration leads to an increase in sulphuric acid production and more experimental work is needed in order to elucidate the relationship between the sulphuric acid production and the iron pyrite reduction. The result strongly suggests that the increased rate of iron pyrite leaching exhibited by Thiobacillus ferrooxidans, is not simply an effect due to the elevated temperature but rather to the combination of temperature and other factors which affect the catalytic efficiency of the process.Bacteria activity appears to be favoured by the higher temperatures 40°-45°C and higher slurry concentrations between 20% - 30% w/v.

ACKNOWLEDGEMENTS

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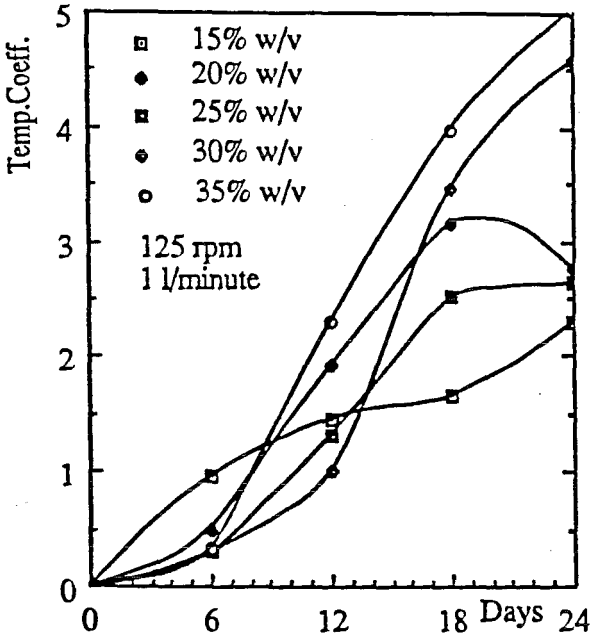

Figure 2: Profile temperature coefficient at 35 to 40 degree Celsius

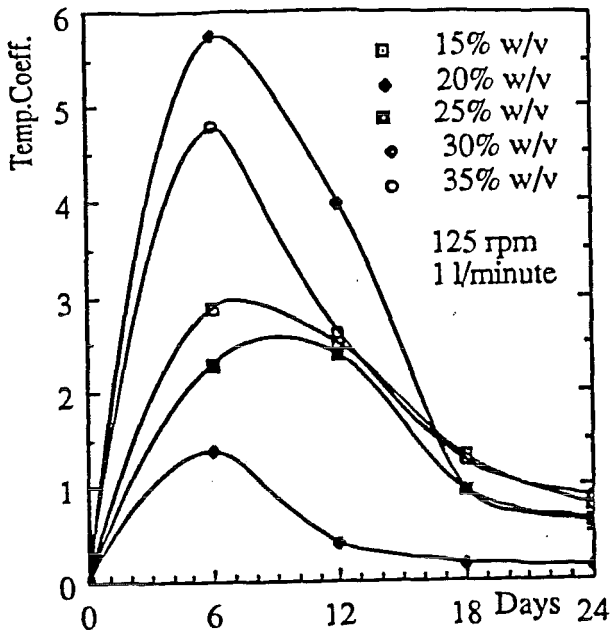

Figure 3: Profile temperature coefficient at 40 to 45 degree Celsius

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THE CORRELATION OF PYRITIC SULPHUR REMOVAL WITH PH VALUES FROM COAL IN WATER SLURRIES CONTAINING MIXED ACIDOPHILIC CULTURE

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ABSTRACT

Up to 98% removal of pyritic sulphur from coal slurries in water using mixed acidophilic microbial cultures dominated by Thiobacillus ferroxidans has been demonstrated in laboratory scale continuous stirred reactors. In this study the calculation of pyritic sulphur removal from coal was based on the chemical analysis of the sulphur content before and after microbial desulphurisation. The analytical procedure is laborious, time consuming and tends to be expensive in terms of labour & materials.

In order to obtain more prompt information, another measurable property which can be used to monitor the rate of pyritic sulphur conversion is suggested.
The correlation between pH value and concentration of pyritic sulphur has been experimentally determined and an equation incorporating solution pH & pyritic sulphur concentration in the coal phase has been developed for coal slurries.

The general form of this equation is:

$$C_t/C_o = \alpha + \beta * (pH_t/pH_o)$$

This pH equation allows us to monitor the progress of the coal desulphurisation on line using continuous pH measurement of the medium.

Based on this equation the pyritic sulphur removal from coal in water slurries can be accurately predicted. For example, in experimental runs with air flow rate 1.5 litre/minute, stirrer rotation 125 rpm, slurry concentration within the range of (15-35)% w/v and temperature range (30-45)° Celsius, the average absolute discrepancy between predicted & experimental data was 3.75%.

INTRODUCTION

Most of the investigations in microbial coal desulphurisation resort to analysis of dissolved total iron & sulfate to permit calculation of the overall removal of the pyritic sulphur from coal.
In this study of coal desulphurisation by using mixed cultures, Thiobacillus ferrooxidans dominant, the calculation of the pyritic sulphur conversion is also based on the standard analysis of the sulphur content before and after desulphurisation. This analysis has been laborious and time consuming.
In order to obtain more immediate information from the process, a more suitable indicator which can be measured on line was sought..
Evaluation of the pH values derived from the experimental runs on this study indicated that the pH changes during the process were linear with the pyritic sulphur content, where by a decreasing concentration of pyritic sulphur causing the pH value of the medium to decrease.
The correlation between the pH value & concentration of pyritic sulphur has been determined and as a result an equation incorporating pH & pyritic sulphur concentration has been developed.which has a general form as $Ct/Co = \alpha + \beta * (pHt/pHo)$.

This pH equation can be used to calculate the amount of the pyritic sulphur removal during the process based on the pH data derived from the medium. This equation allows the progress of the coal desulphurisation to be monitored on line as long as the pH of the medium is measured. This pH equation is only a simplified estimation for the rate of pyritic sulphur oxidation.
The major advantages of this method are that it is quick and gives a result accurate enough, for practical purposes.

LITERATURE REVIEW

Previous investigations of microbial pyritic sulphur decomposition have been concerned with the release of either iron or sulfate.
In this study only one of these two elements was measured and decomposition was assumed to proceed stoichiometrically according to the reaction:

$$2FeS_2 + 7.5O_2 + H_2O \rightarrow Fe_2(SO_4)_3 + H_2SO_4$$

Several publications dealing with this subject have since appeared in the literature.
For example, **Kargi (1)** calculated the pyritic sulphur removal by using the stoichiometric relationship between the sulphur & iron content of pyrite (i.e. S pyrite/Fe pyrite = 1.143).
On the other hand, **Andrews, C.F. & Maczuga, J. (2)** measured both iron & sulfate content, because they found there was a misleading picture of the coal desulphurisation rate which is encountered if only iron release is measured.Limited information is available on the use of other methods to calculate the overall removal of the pyritic sulphur from coal.The possibility of the use of other methods was reported by **Huber et al. (3)** who published comparable results applying the different methods (total iron, sulfate & oxygen consumption). He proved the suitability of the microbial oxygen consumption rate based on gas phase analysis as a measure for pyritic sulphur oxidation.
Hone, H.J. et al. (4) employed the redox potential to calculate the pyritic sulphur conversion in his experiment with tank reactor. In his experiment, the pyritic sulphur conversion process by pure Thiobacillus ferroxidans cultures, an increase in ferric iron concentration in the medium caused an increase in the redox potential which varied directly with the conversion of pyritic sulphur, in the case of one coal and one slurry density.
Klein, J., et al. (5) mentioned that calculations based on the analysis of the sulphur content before and after desulphurisation are scarce. Since the analytic methods of sulphur content are laborius, time consuming and therefore expensive.

RELATION BETWEEN pH VALUE AND PYRITIC SULPHUR CONCENTRATION

The concentration-time plots of pyritic sulphur in the coal phase and pH of the medium versus time show that increasing amounts of pyritic sulphur removal caused increasing acidity of the coal-water medium .
Figure 1 shows a typical relationship of pyritic sulphur removal and pH value in this microbial coal desulphurisation process.Based on this information it can be assumed that the pH value indicates the extent of the pyritic sulphur oxidation or vice-versa.
In order to derive a correlation between the pH value and the pyritic sulphur content, the data of pH obtained from the experimental runs and pyritic sulphur based on the analysis of coal samples have been collected and examined.
Data Ct/Co and pHt/pHo derived from this experiment runs have a similar pattern which show a decrease trend with respect to time.

Data Ct/C^{o} & pHt/pH^{o} used for this correlation were derived from the experimental runs within the range of temperature $(30\text{-}45)^{o}$ Celsius and slurry concentration in the range (15-35)% w/v at different reactor conditions:

1. air flow rate 1 litre/minute and stirrer rotation 125 rpm
2. air flow rate 1.5 litre/minute and stirrer rotation 125 rpm and
3. air flow rate 1.5 litre/minute and stirrer rotation 175 rpm.

The correlation is calculated in the case of one type of coal, one temperature and one slurry concentration.

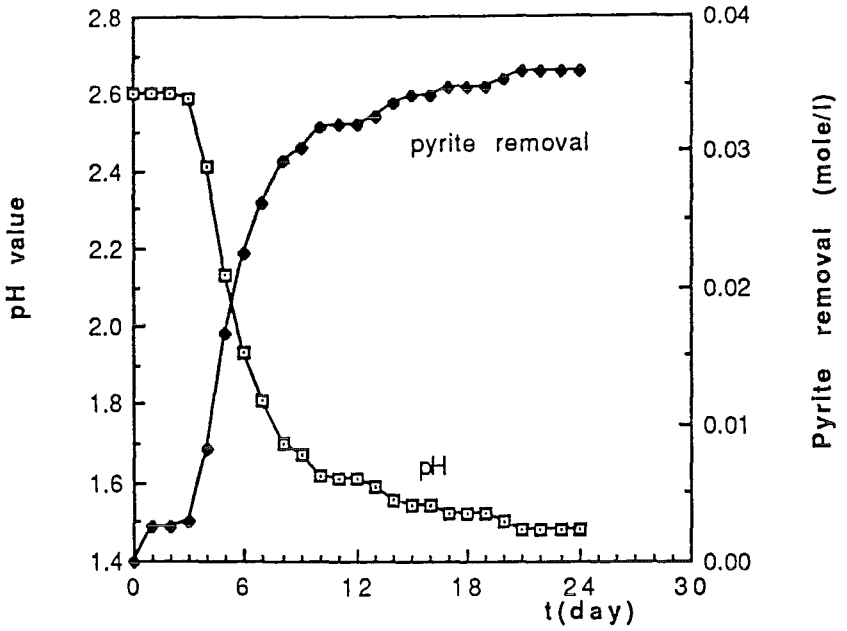

Figure 1 : Profile pyrite removal and pH value

RESULTS & DISCUSSION

The distribution of these data allow the development of a correlation between pH and pyritic sulphur concentration indicated by plotting pHt/pHo versus Ct/Co.
A typical correlation between pHt/pHo and Ct/Co in this study is shown in figure 2. This correlation is for an experimental run with air flow rate 1.5 litre/minute and stirrer rotation 125 rpm at 40º Celsius and 30% w/v of slurry concentration.

The general form for this relation is as follows:

$$Ct/Co = \alpha + ß * (pHt/pHo)$$

where:
Ct is concentration of pyritic sulphur at time t (day);
Co is initial concentration of pyritic sulphur (mole/litre);
pHt is pH value medium at time t and pHo is the initial pH of the medium.

The constants α and ß for each pH equation obtained from the fitting of data are shown in tables 1; 2 ; and 3.

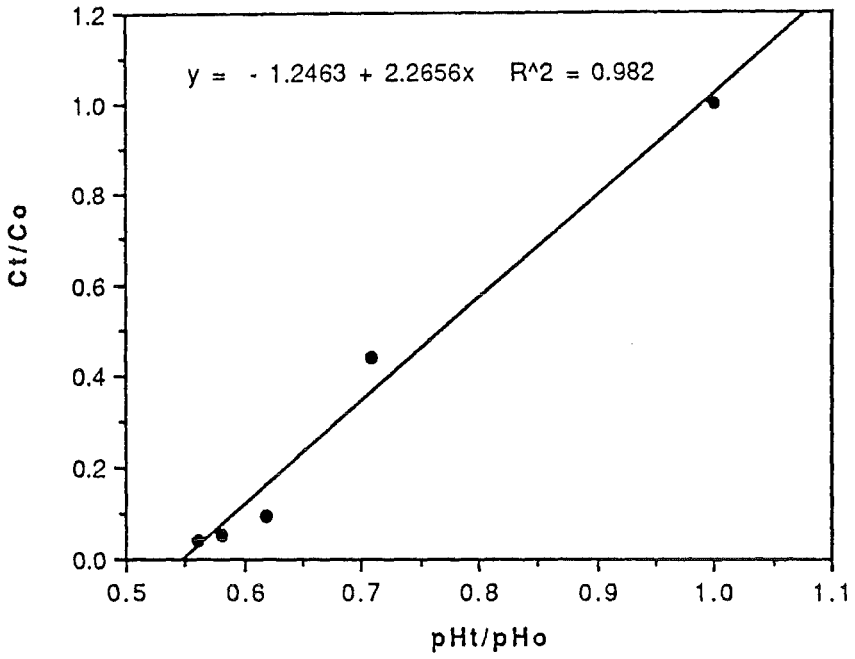

Figure 2 : Plot Ct/Co versus pHt/pHo for experiment at 40°C, 1.5 l/min., 125 rpm, 30% w/v.

Table 1: Constants α & ß for pH equation in microbial coal desulphurisation with air flow rate 1.0 litre/minute & stirrer rotation 125rpm

temp. (°C)	s. conc. (% w/v)	Constants for pH equation		R squared	R
		α	ß		
30	15	-1.0106	+1.8509	0.808	0.899
	20	-1.0053	+1.8870	0.895	0.946
	25	-0.9026	+1.7196	0.818	0.904
	30	-1.1618	+2.1383	0.978	0.989
	35	-0.4137	+1.4032	0.822	0.907
35	15	-1.9835	+2.9500	0.981	0.990
	20	-1.7882	+2.7497	0.982	0.991
	25	-1.6367	+2.6253	0.985	0.992
	30	-1.6061	+2.5823	0.991	0.995
	35	-1.3046	+2.2919	0.981	0.990
40	15	-1.1767	+1.9655	0.800	0.894
	20	-0.9181	+1.7267	0.822	0.907
	25	-1.0459	+2.0078	0.994	0.997
	30	-0.9167	+1.8034	0.946	0.973
	35	-0.8549	+1.7295	0.926	0.962
45	15	-1.4648	+2.4165	0.981	0.990
	20	-1.4855	+2.4140	0.861	0.928
	25	-1.1628	+2.1942	0.993	0.996
	30	-1.1459	+2.1695	0.995	0.997
	35	-0.8615	+1.8835	0.947	0.973

Table 2: Constanta α & ß for pH equation in microbial coal desulphurisation with air flow rate 1.5 litre/minute & stirrer rotation 125rpm

temp. (ºC)	s. conc. (% w/v)	Constants for pH equation		R squared	R
		α	ß		
30	15	-2.0644	+2.9293	0.907	0.952
	20	-1.7204	+2.5842	0.903	0.950
	25	-1.8014	+2.7497	0.985	0.992
	30	-1.5795	+2.5633	0.992	0.996
	35	-1.6167	+2.5992	0.993	0.996
35	15	-1.9614	+2.9149	0.985	0.992
	20	-1.6832	+2.6984	0.997	0.998
	25	-1.4813	+2.4538	0.991	0.995
	30	-1.4503	+2.4539	0.999	0.999
	35	-1.3532	+2.2972	0.967	0.983
40	15	-1.8084	+2.7866	0.984	0.992
	20	-1.5284	+2.4875	0.976	0.988
	25	-1.3902	+2.3801	0.995	0.997
	30	-1.2463	+2.2656	0.982	0.991
	35	-1.1795	+2.2090	0.987	0.993
45	15	-1.4285	+2.4140	0.991	0.995
	20	-1.4054	+2.4306	0.976	0.988
	25	-1.1149	+2.1381	0.989	0.994
	30	-1.0213	+2.0436	0.988	0.994
	35	-1.0413	+2.0406	0.996	0.998

From the tables, the constants α and ß for each set of experimental conditions are as follows:

1. For experimental runs with conditions:
   air flow rate 1.0 litre/minute & stirrer rotation 125 rpm
   temperature range: 30-45º C
   slurry concentration: 15-35% w/v

   The constant α is within the range (-0.9026 to -1.9835) and
   ß is within the range of (+1.4032 to +2.9500) with
   the correlation coefficient (R) in the range (0.894 -0.997).
2. For experimental runs with conditions:
   air flow rate 1.5 litre/minute & stirrer rotation 125 rpm
   temperature range: 30-45º C
   slurry concentration: 15-35% w/v

   The constant α is within the range: (-1.0213 to -2.0644) and
   ß is in the range of (+2.0406 to +2.9293) with
   the coefficient correlation (R) in the range (0.950-0.999).
3. For experimental runs with conditions:
   air flow rate 1.5 litre/minute & stirrer rotation 175 rpm
   temperature range: 30-45ºC
   slurry concentration: 15-35% w/v

   The constant α is within the range of (-1.0100 to -2.3761) and
   ß is in the range (+2.0206 to 2.8800) with
   the coefficient correlation (R) in the range (0.918-0.999).

With these pH equations the pyritic sulphur removed from the coal at time t can be estimated based on the pH of the medium at t time.

COMPARISON BETWEEN PREDICTED AND EXPERIMENTAL DATA

The comparison of the predicted & experimental values of pyritic sulphur (Ct/Co) for different experimental conditions are shown in figures 3; 4; and 5.
From these figures, the plot of predicted Ct/Co against actual Ct/Co shows that the points are clustered about the expected line of fit. However, some particular data points do not fit the line well.

The average absolute percentage discrepancies between the predicted and experimental data for:
1. Experiment runs at air flow rate 1.0 litre/minute & stirrer rotation 125 rpm is 6.21%
2. Experiment runs at 1.5 litre/minute volume of air & 125 rpm stirrer rotation is 3.75 %
3. Experiment runs at 1.5 litre/minute air flow & 175 rpm stirrer rotation is 4.10 %.

Figure 6 shows an example of the comparison between experimental & predicted pyrite removal calculated by using this pH equation. The figure illustrates the result for the experiment run at 30º, 35º, 40º and 45ºC with air flow rate 1.5 litre/minute and stirrer rotation 175 rpm for 25% w/v of slurry concentration. This shows close coincidence of the experimental points with the predicted curve.

Table 3: Constants α & ß for pH equation in microbial coal desulphurisation with air flow rate 1.5 litre/minute & stirrer rotation 175 rpm

temp. (ºC)	s. conc. (% w/v)	Constants for pH equation		R squared	R
		α	ß		
30	15	-2.3761	+3.2668	0.950	0.975
	20	-1.6213	+2.6097	0.843	0.918
	25	-1.7995	+2.7842	0.982	0.991
	30	-1.7319	+2.7903	0.987	0.993
	35	-1.5275	+2.5435	0.995	0.997
35	15	-1.9345	+2.8800	0.983	0.991
	20	-1.5713	+2.5601	0.996	0.998
	25	-1.4392	+2.4629	0.993	0.996
	30	-1.4584	+2.5250	0.976	0.988
	35	-1.1955	+2.1599	0.993	0.996
40	15	-1.5735	+2.5333	0.980	0.990
	20	-1.4162	+2.4082	0.993	0.996
	25	-1.3081	+2.3078	0.999	0.999
	30	-1.1170	+2.1646	0.957	0.978
	35	-1.0836	+2.1440	0.946	0.973
45	15	-1.4243	+2.4510	0.979	0.989
	20	-1.2444	+2.2354	0.996	0.998
	25	-1.0100	+2.0206	0.996	0.998
	30	-1.0647	+2.1127	0.966	0.983
	35	-1.0908	+2.1320	0.986	0.993

CONCLUSION

The prediction of the pyritic sulphur oxidation is demonstrated using an equation based on the pH values derived from experimental runs for microbial coal desulphurisation by using a mixed culture, which is Thiobacillus ferrooxidans dominant.

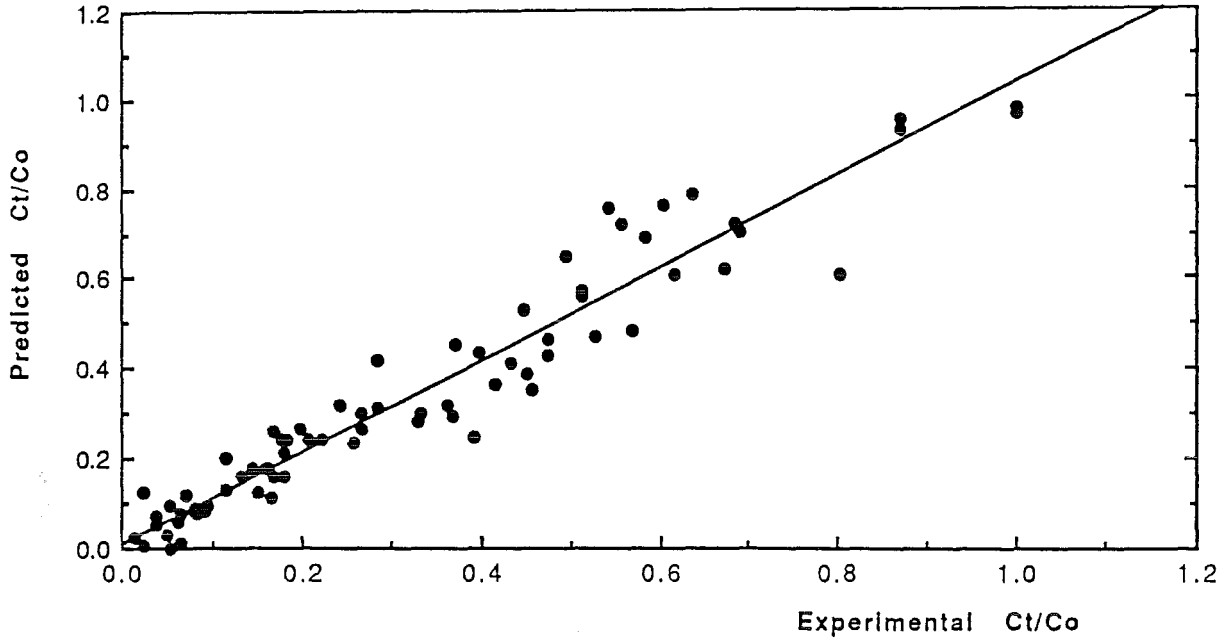

Figure 3: Plot predicted vs experimental Ct/Co for experiment at 1.0 l/min., 125 rpm, 15-35% w/v and 30-45ºC.

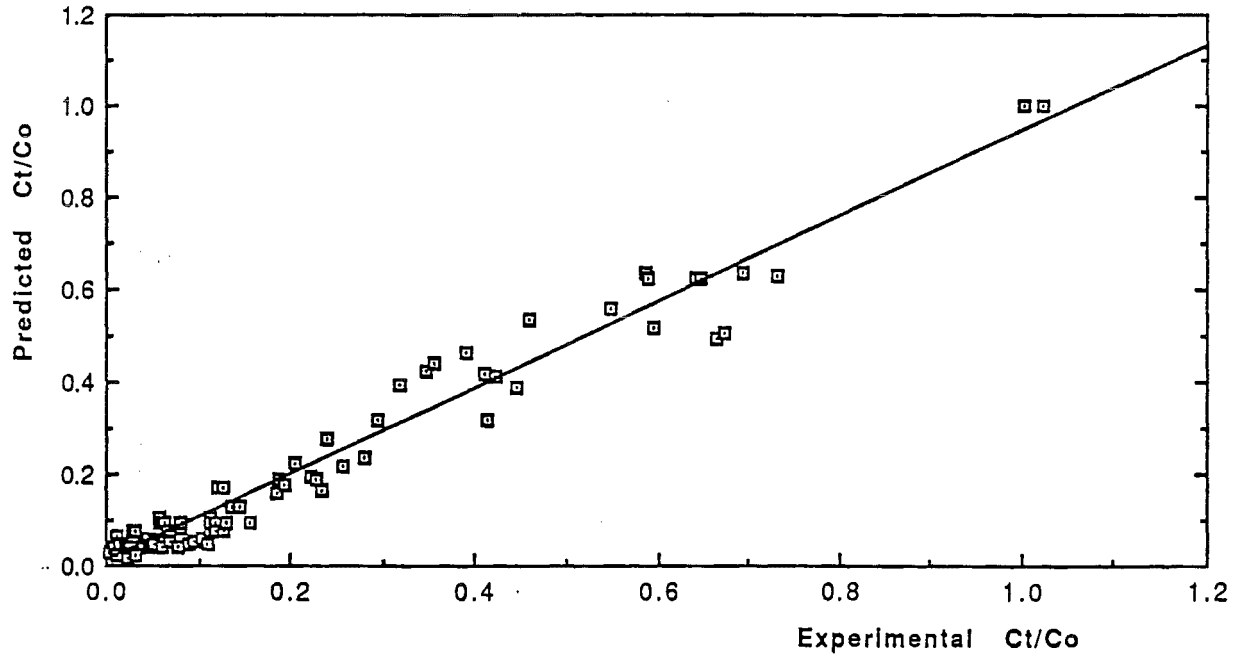

Figure 4: Plot predicted vs experimental Ct/Co for experiment at 1.5 l/min., 125 rpm, 15-35% w/v and 30-45º C.

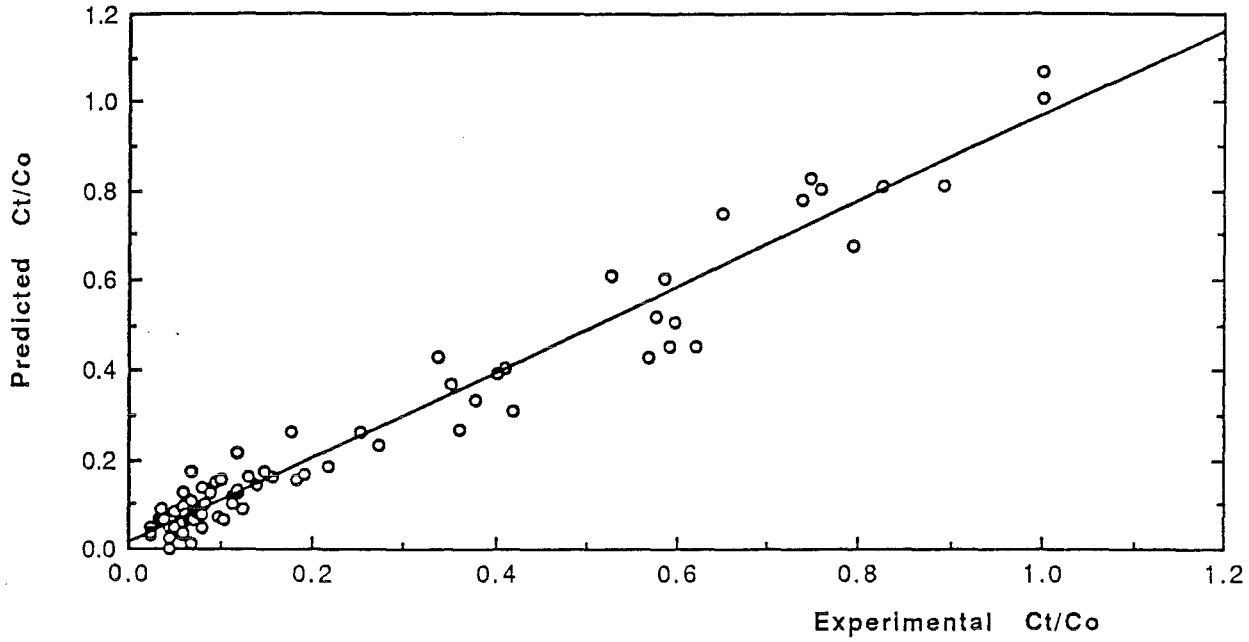

Figure 5: Plot predicted vs experimental Ct/Co for experiment at 1.5 l/min., 175 rpm, 15-35% w/v and 30-45ºC.

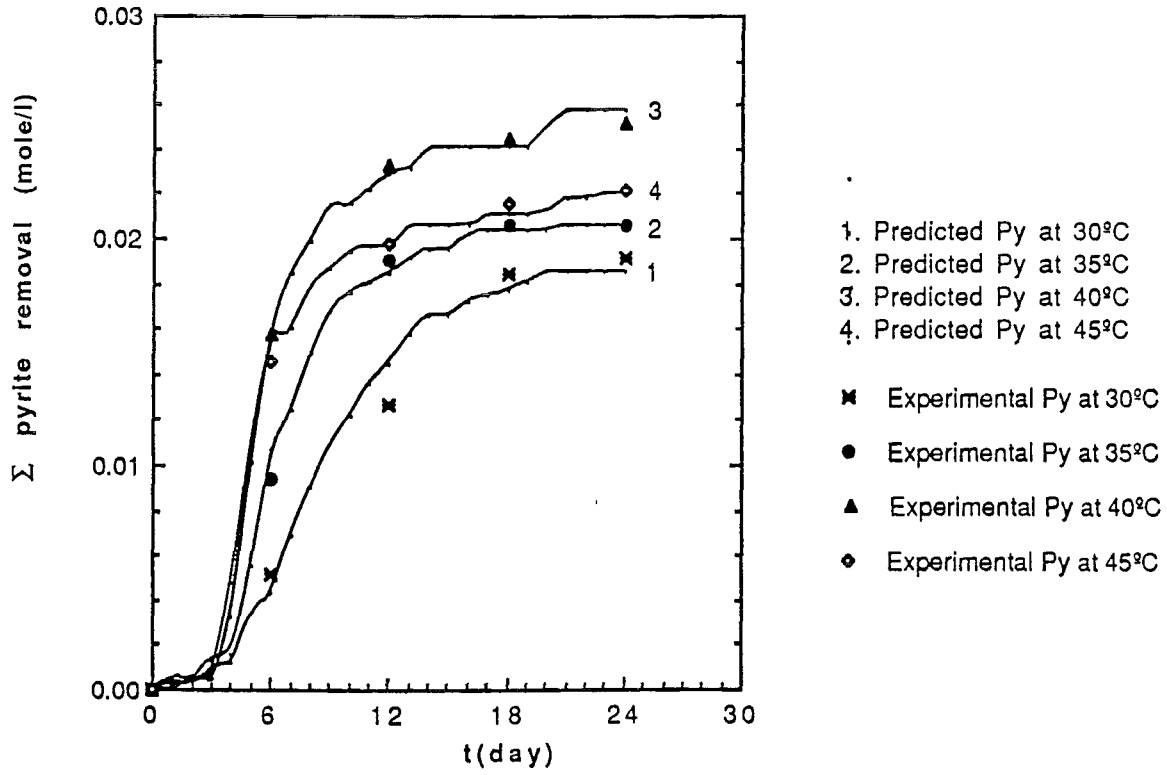

Figure 6: Graph predicted & experimental Σ Pyrite removal for experiment at 1.5 litre/minute & 175 rpm, 25% w/v, temperature range: 30-45º Celsius.

The pH equations in this study give reasonable estimation of the concentration of pyritic sulphur where the absolute percentage discrepancies between the predicted and experimental data is as following:
a. 6.21% for the experiment run at air flow rate 1.0 litre/minute & stirrer rotation 125 rpm
b.3.75% for experiment at 1.5 litre/minute & 125 rpm and
c. 4.10% for experiment runs at 1.5 litre/minute & 175 rpm.
This method allows us to obtain immediate information from the experimental run by monitoring the pH value of the liquid medium.

SYMBOLS:

α = constant
β = constant
Ct = concentration of pyritic sulphur (mole/litre) at time t (day)
Co = initial concentration of pyritic sulphur (mole/litre)
pHt = pH value of medium at time t
pHo = initial pH of the medium

ACKNOWLEDGEMENTS

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PART II

MICROBIAL ORGANIC SULPHUR REMOVAL

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PART II

MICROBIAL ORGANIC SULPHUR REMOVAL

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ABSTRACT

The use of microbes to remove organic forms of sulphur in coal has been studied. Mixed acidophilic cultures (designated HB1 and HB2), isolated by a simple selection culture procedure have been shown to be capable of removing organic sulphur from coal.

Preliminary identification showed that the cultures, HB1 and HB2, were a consortium of microorganisms, consisting of rod shaped *Bacillus* strains, species of *Aspergillus* and *Penicillium* fungi together with unidentified microbes. Experimental results gave good reason to suppose that these consortia collectively had the capacity to degrade both forms of sulphur from coal. The results indicated that HB1 and HB2, probably contained *Thiobacillus ferrooxidans* since they were also capable of removing pyritic sulphur from coal. There were two entirely different groups of microorganisms; one group of microorganisms attacked the pyritic sulphur best at 37°C, whilst the other attacked organic sulphur and had an optimum temperature of 47°C.

Characterization of the cultures indicated that the rate of organic sulphur removal depended on the particle size and the type of coal. Decreasing particle size from 152 μm to 63 μm resulted in an increase of the rate of organic sulphur removal by factors of 2.0 and 2.3, for cultures HB1 and HB2, respectively. Further decrease in coal particle size could lead to higher rates of organic sulphur removal, although economic considerations would become increasingly important.

Although it has been demonstrated that organic sulphur from different types of coal (pretreated Benneydale coal; "fresh" Benneydale coal and New Creek coal) could be removed by microbial action the process was not clearly understood. The accessibility to the interior of the coal was an important factor influencing the rate and amount of organic sulphur removal by microbes. The more compact the coal, the less accessible it was to microbial attack, making degradation correspondingly more difficult.

CHAPTER 1

INTRODUCTION

1.1. GENERAL INTRODUCTION AND OBJECTIVES OF THE WORK

1.1.1. GENERAL INTRODUCTION

The possibility of applying biotechnological methods to remove pyritic and organic sulphur from fuels, especially coal, has attracted more interest from researchers in the last 50 years. Most research on the biological processing of coal has been directed to pyritic sulphur rather than organic sulphur removal. Despite increased research worldwide organic sulphur removal by microbiological agents i.e. biodegradation is still at the basic research stage. Biodegradation is often limited by the metabolic rate of the microorganisms and the stability of their constituent enzymes and other biomolecules.

Use of microorganisms offers a number of potential advantages.

Firstly, microorganisms are not consumed but multiply.

Secondly, they are effective at ambient temperature and pressure, so that they offer the potential of low-energy and low-cost processing.

Thirdly, the lack of high temperature, pressure and requirement for addition of (often) hazardous materials, means that biological processes offer fewer pollution problems.

Monticello and Finnerty (1985) indicated that the organisms which were capable of degrading organic sulphur compounds were most commonly heterotrophic, soil bacteria from the genera *Pseudomonas, Arthrobacter, Acinetobacter* and *Rhizobium* . They required pH conditions around 7

and obtained their carbon and energy for growth from organic compounds present in their environment. In contrast, the acidophilic *Thiobacillus ferrooxidans* which attacked pyritic sulphur required acidic conditions (see part I of this thesis). A bioprocess which is intended to remove both types of sulphur would require different operating conditions; in this case at least a change of pH and growth medium will be needed.

In an attempt to develop an acidic process in which both forms of sulphur would be degraded, acidophilic cultures which are capable of removing organic sulphur are more preferable, because so far no organisms that attack pyritic sulphur at neutral pH have been isolated. A more reasonable approach is to isolate acidophilic heterotrophic microbes which are capable of degrading organic sulphur (Roberto *et al.* 1991).

1.1.2. OBJECTIVES OF THE WORK

The aims of this study were as follows:

(i). To conduct an up to date review of the literature on microbial organic sulphur removal. Also to examine coal structure and organic sulphur forms since these might affect microbial activity.

(ii). To isolate acidophilic microbes with the capability of removing organic sulphur from coal.

(iii). To characterize the isolated microorganisms, paying particular attention to factors that likely affect the desulphurisation process e.g. particle size, temperature, nutrients.

1.2. CURRENT KNOWLEDGE OF MICROBIAL ORGANIC SULPHUR REMOVAL

Knowledge of the biochemical reactions involved in the removal of organic sulphur from coal has been derived from investigations into the desulphurisation of model compounds. Most of these investigations have focused on metabolic pathway analysis for dibenzothiophene (DBT) and not for coal. The difficulty of using coal as the substrate for microbial coal desulphurisation has necessitated the use of DBT. DBT is considered to be an ideal model compound representing typical organic sulphur structures found in coal. However, some experts still doubt whether the microbes which work well with DBT would also produce the same result when working with different types of coal (Bos, 1990). The validity of this model compound has never been clearly established.

Many articles on microbial metabolism of organic sulphur containing compounds have been published since Yamada *et al.* in 1968 used reduced sulphur containing model compounds such as DBT in enrichment studies to develop a bacterial desulphurisation system for crude oil. They isolated two bacteria, *Pseudomonas jianni* and *Pseudomonas abikonensis* which could convert DBT into water soluble compounds.

Kodama *et al.* (1970) were the first to identify some of the degradation products of DBT. They reported the identification of products from degradation of DBT by *Pseudomonas jianni* and *Pseudomonas abikonensis* . The incubation was aerobic, at 28°C on a shaker at 110 rpm for seven days, in a liquid meat extract medium containing 1 gram DBT per litre of medium, at an adjusted pH of 7.3 and with an inoculum size of 5% by volume. The fermentation broth was filtered, chromatographed and five organic compounds isolated. The organic compounds were subjected to different identification procedures such as infra-red and nuclear magnetic resonance spectra analyses. These tests identified three compounds: 3-hydroxy-2-formyl-benzothiophene, dibenzothiophene-5-oxide and 4(2-(3-hydroxy)-thionapthenyl-2-oxo-3-butenoic acid (see figure 1. 1). Two other products could not be identified.

The pathway in figure 1. 1 was proposed by Kodama *et al.* (1973) and confirmed by other works; Hou and Laskin (1976); Malik and Klaus (1976); Laborde and Gibson (1977).

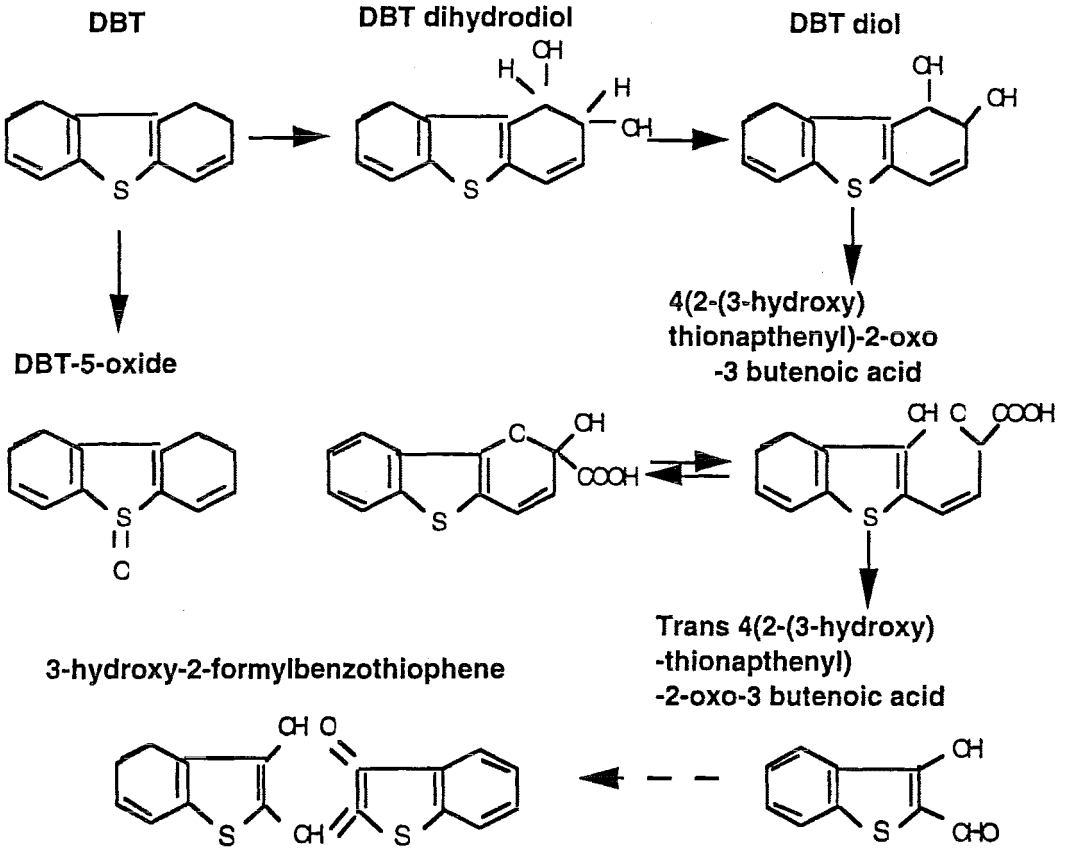


Figure 1. 1. Biodegradation pathway of DBT (from Kodama *et al.* 1973)

The study of Babenzien *et al.* in 1979 showed that another model compound, dibenzylsulphide (DBS) could be attacked by a mixed bacterial culture wherein DBS was utilized as a carbon and energy source.

One study, conducted in 1979 by Chandra *et al.* indicated that a mixed bacterial culture isolated from soil samples, enriched on DBT, could remove up to 20% of the organic sulphur present in Indian Baragoi coal after 10 days of incubation in a laboratory shaker at 30°C.

An interesting development involves the thermophilic bacteria, *Sulfolobus brierleyi,* described by Bhattacharyya *et al.* (1981). This organism can be adapted to preferentially metabolize either organic or pyritic sulphur. As much as 45% of organic sulphur could be removed in coal that contained approximately equal amounts of organic and pyritic

sulphur whilst the pyritic sulphur content remains unchanged. For other coal samples, the same bacterial culture removed 95% of pyritic sulphur with no reduction of organic sulphur.

Other studies by Kargi and Robinson (1982a, b) used a thermophilic archaebacterium (*Sulfolobus acidocaldarius*) growing at low pH (2-3) and high temperatures (50-80)^{o}C to remove both organic and pyritic sulphur from coal. The experiment, using sterilized and acid pretreated coal, resulted in the removal of 19% of the organic sulphur within 28 days at 75^{o}C. The desulphurisation experiments were carried out in a medium with different amounts of a coal (containing 4% total sulphur; 1.9% organic sulphur and particle size of 104-107 μm). Another study (Kargi, 1984) using the same culture, confirmed that about 65% of the initial sulphur in DBT was oxidized to sulphate within 28 days at 70^{o}C and pH 2.5. A slurry of 15% coal was found to be optimal (Kargi and Robinson, 1986). The observation that *Sulfolobus acidocaldarius* attacked the organic sulphur in coal was supported by further studies using model compounds. Other model compounds, such as thioxanthene and thianthrene were also utilized by this *Sulfolobus acidocaldarius* as sole carbon and energy source (Kargi, 1987). No experiments on the degradation pathways of these compounds have been described. However, Kilbane (1989) working with *Sulfolobus acidocaldarius* were unable to confirm Kargi's 1987 results.

Another study conducted by Gökcay and Yurteri in 1983 showed that a mesophilic strain of *Thiobacillus ferrooxidans*, designated TH1, cultivated on a sulphate and yeast extract containing medium at 50^{o}C and pH of 3.0 could remove 50 to 56 % of the organic sulphur and 90 to 95 % of pyritic sulphur in Turkish lignite over a 25 day incubation period .

In 1985, Isbister and Doyle, reported that a soil isolate, *Pseudomonas* mutant (ATCC 39381), was capable of removing organic sulphur from coal. The isolate was adapted to the presence of DBT (100-1000 mg/l) in a medium containing sulphur and citric acid. Cultures were selected in a mineral medium supplemented with benzoate and DBT (200 or 400 mg/l). The mutant designated CB1 ("Coal Bug 1") had shown the ability to attack DBT which was then converted to biphenol. CB1 did not break the carbon ring structure of DBT. Further experiments showed that CB1

was able to attack other forms of organic sulphur compounds such as n-octyl sulphide, benzylmethyl sulphide, thioanisle and 1-benzothiophene. Studies using DBT indicated that CB1 appeared to be most effective in removing thiophenic sulphur on a laboratory scale. CB1 removed 18-47% of organic sulphur in coal at residence times of 9-18 hours depending on the coal type, particle size, initial organic sulphur content and other, as yet unidentified, parameters (Isbister and Kobylinski, 1985). The coal used had 90% of its total sulphur has been removed. Besides laboratory studies, a continuous microbial system that could process 5 kgs per day of coal was developed. Organic sulphur could be reduced 10 to 29 percent weight, depending apparently, on the coal type and other unidentified parameters. However, the laboratory (Arctech Inc., formerly Atlantic Research Corporation) where CB1 was originally isolated now no longer possesses a culture with desulphurisation activity. Arctech Inc. has also isolated another microorganism designated CB2, an *Acinetobacter,* that has shown the ability to oxidize model aryl sulphide compounds such as diphenyl sulphide (DPS) and benzyl phenyl sulphide (BPS). This microorganism was also tested on various coals with mixed success. Up to 30% removal of coal organic sulphur tested was achieved. Again the variation in effectiveness seemed to depend, amongst other factors, on coal type. Like CB1, CB2 had a negligible effect on the removal of pyritic sulphur. Both organisms CB1 and CB2 could release organic sulphur from coals, but some instability in this trait has been observed (Couch, 1987). Recently, Arctech. developed a semi-continuous flow airlift of DBT degrading organisms, designated CB3, which was isolated using a selectostat bioreactor. This CB3 consortium used DBT as sulphur source. Partial characterization of CB3 revealed that there were possibly 10 different organisms in the consortium which indicated that the metabolism of DBT was a complex multi step enzymatic conversion (Harding *et al.* 1990). In this pathway the oxidation of DBT to DBT-sulphoxide (DBT-5-oxide) was a reversible reaction with little oxidation of sulphoxide taking place. Biphenyl-sulphonate, although not detected as an intermediate, is postulated as an intermediate. DBT-sulphone, monohydroxybiphenyl, dihydrobiphenyl, and biphenyl were produced as intermediates. Ultimately, CO_2 was the final product.

Another study, Van Afferden *et al.* (1988) showed a mixed culture consisting of *Alcaligenes denitrificans* and *Brevibacterium* species. Both

were non-motile, capable of utilizing DBT as sole sulphur for growth while benzoate was used as carbon source. Degradation of DBT was in the range of (79.5-91.8)%. Results indicated that bacterial growth and DBT degradation remained almost constant, in spite of the presence of varying concentrations of sulphate (0-100 μm). This indicated that the first step in DBT degradation was not repressed by sulphate. The results indicated that sulphur elimination proceeds via the corresponding sulphoxide and sulphone.

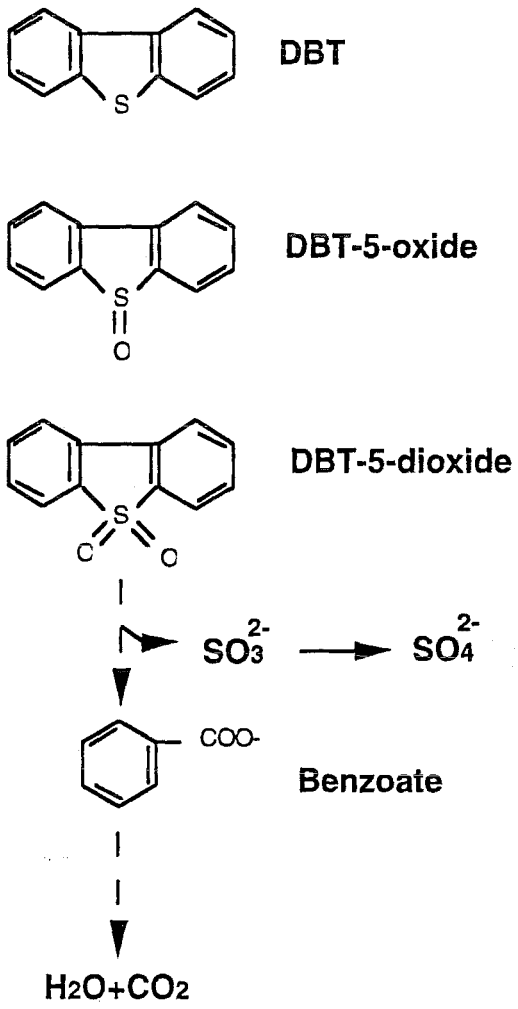


Figure 1. 2. Proposed pathway for the degradation of DBT by *Brevibacterium* sp. (from Van Afferden *et al.* 1990)

Further studies by Van Afferden *et al.* (1990) on the *Brevibacterium sp.* showed that in the presence of the growth factor, thiamine, DBT utilization was initiated by an oxidative attack on the sulphur hetero-atom. Moreover, the release of sulphate in stoichiometric amounts indicated that the thiophene ring was cleaved and DBT was mineralized

completely to water, CO_2 and SO_4^{2-}. During degradation of DBT, sulphite was released and subsequently oxidized to sulphate. Three metabolites of DBT were identified as DBT-5-oxide, DBT-sulphone and benzoate. End products of this pathway were H_2O and CO_2 (see figure 1. 2).
This proposed metabolic pathway for the total mineralization of DBT by *Brevibacterium sp.* represents an alternative to the "classical" pathway of partial degradation of DBT established by several authors (Kodama *et al.* 1973; Hou and Laskin, 1976; Laborde and Gibson, 1977).

Klubek *et al.* (1988) isolated some pure bacterial strains which were capable of utilizing benzene sulphonic acid, cystine, and several thiophenes. The microbial attack on acid treated and non acid treated coal samples (Illinois no 5, 60 mesh) resulted in a decrease of total sulphur varying from 0.0 to 7.0%.

Efforts in this area by the Institute of Gas Technology , Chicago, Illinois, USA, have recently resulted in the evolution of a culture with desulphurising activity which was accelerated by chemical mutagenesis (Kilbane and Maka, 1987 and Kilbane, 1989). The Institute of Gas Technology has identified a strain of microorganism designated IGST7 that, when grown on several carbon substrates, was capable of degrading a wide variety of model sulphur compounds, including DBT. IGTS7 metabolized DBT to monohydroxyl biphenyl (MHBP) and removed up to 91% of the organic sulphur from Illinois no. 6 coal after 212 days of chemostat operations, although the coal apparently experienced a carbon loss of 39% during this procedure. This result was highly encouraging because it was the first to demonstrate that organic sulphur could be removed from coal by a biological process to a level sufficient to satisfy the Clean Air Act Standard. The major thrust of current work at IGT was towards the isolation of pure cultures from the IGTS7 mixed culture. Two pure cultures were isolated from the mixed IGTS7, which have been identified as *Rhodococcus rodochrous* and *Bacillus sphaericus* species (designated IGTS8 and IGTS9, respectively). These strains have been shown to be unique in their ability to specifically cleave carbon-sulphur bonds. A variety of plasmid containing bacterial cultures that may be useful in genetic analysis of IGTS8 are being evaluated (Kilbane and Bielaga, 1990a, b).

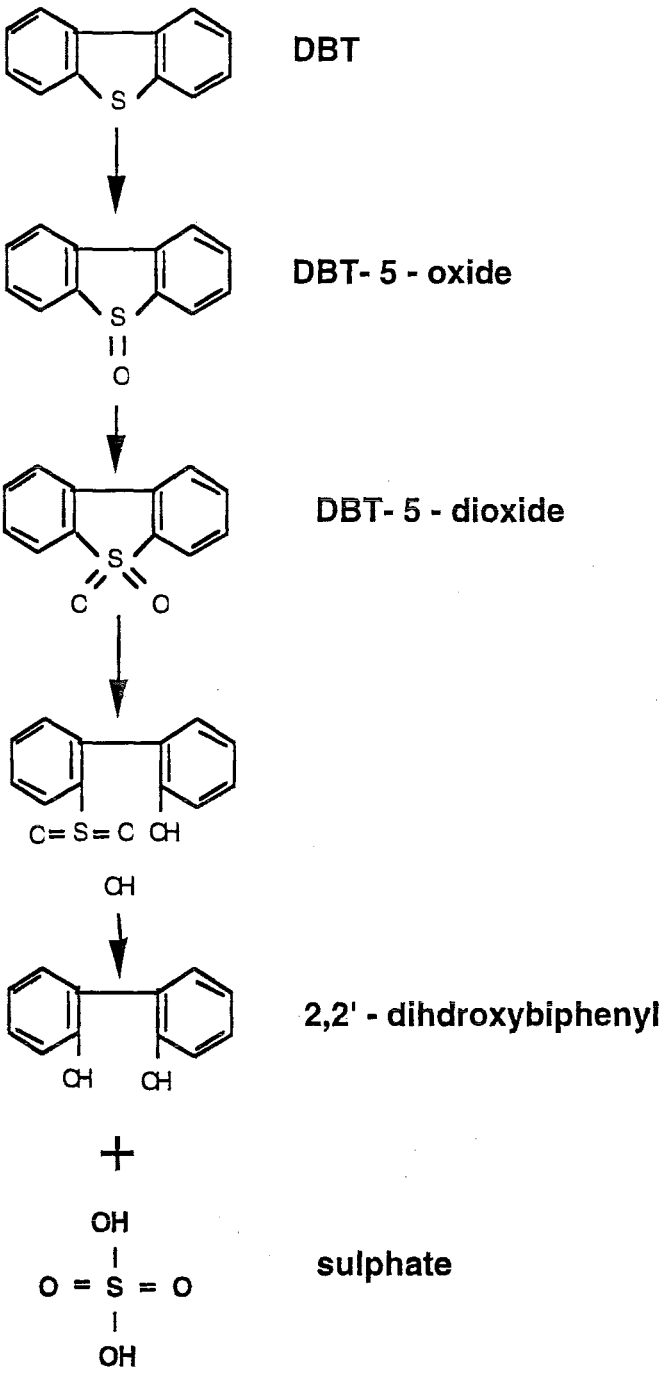


Figure 1. 3. Proposed pathway (4'S') for degradation of DBT (from Kilbane, 1989)

One of their primary concerns about this culture was the stability of the desulphurisation trait of IGTS8. Further experiments will attempt to introduce DNA into IGTS8 by either conjugation or transformation/electroporation and ultimately to clone the genes responsible for the desulphurisation trait of IGTS8. Additional tests showed that IGTS8 also used other model compounds, such as thianthrene ($C_{12}H_8S_2$), thioxanthene ($C_{12}H_8SO$), trithiane ($C_3H_6S_3$) and

a variety of other organo-sulphur compounds as sulphur sources (Kilbane and Bielaga, 1990a, b).
Kilbane (1989) proposed a pathway for the microbial degradation of DBT. In this pathway DBT oxidation passes through DBT-5-oxide, DBT-sulphone and the end products are 2,2-dihydroxybiphenyl and sulphate (see figure 1.3).

Another variation of DBT biodegradation pathway was suggested by Stoner (1989). She worked with non specified microbial strains and demonstrated the formation of DBT-5-oxide and DBT-sulphone as intermediate products and concluded that biphenyl and/or 2,2'-hydroxybiphenyl, and sulphate were the end products (figure 1. 4).

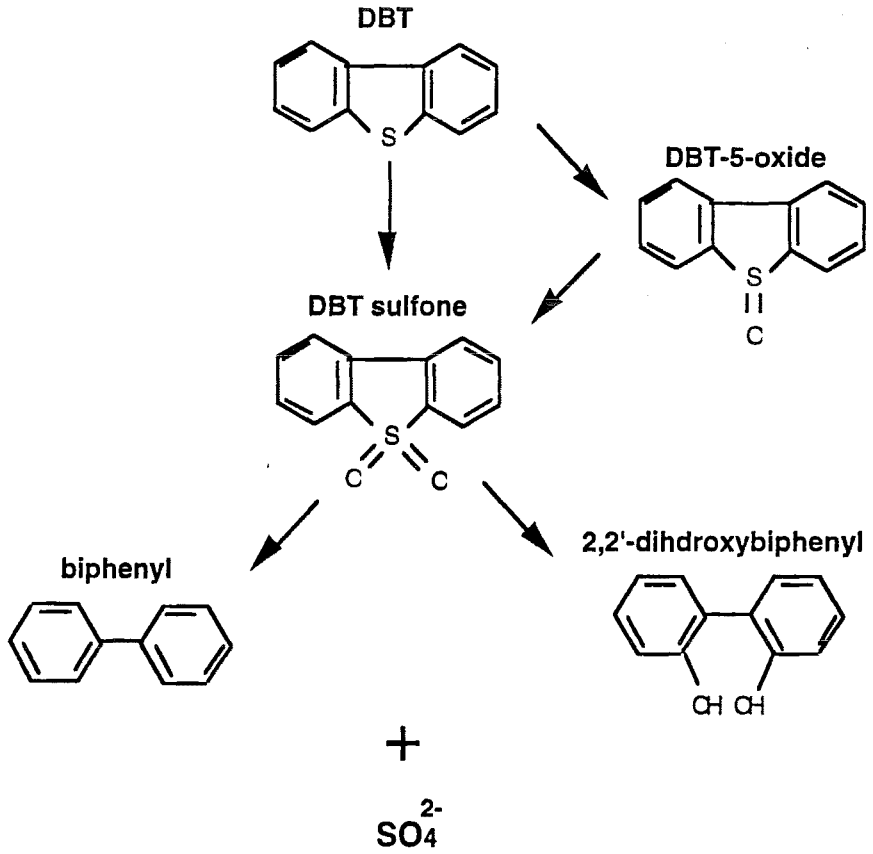


Figure 1. 4. Biodegradation pathway of DBT (from Stoner, 1989)

• A tabulated summary of recent work so far discussed is given in table 1.1.

Table 1.1.
Study of microbial organic sulphur (literature review)

Time	Cultures and Activities	Cond.	References.
1968 1977	*Pseudomonas* strains Pathway degradation of DBT	pH =7 28°C	Hou and Laskin , 1976 Kodama *et al.* 1970,1973 Laborde and Gibson, 1977 Malik and Klaus, 1976 Yamada *et al.* 1968
1979	Mixed cultures, DBS	pH = 7	Bebenzien *et al.* 1979
1979	Mixed cultures , 20% organic sulphur removal from coal	pH = 7 30°C	Chandra *et al* . 1979
1981	Mixed cultures, 45% organic sulphur removal from coal	pH = 3 70°C	Bahttacharyya *et al.* 1981
1982 - 1986	*Sulfolobus acidocaldarius*, 65% reduction of DBT and 19% organic sulphur removal from coal	pH= 2.5 70°C	Kargi and Robinson, 1982a,b, 1984, 1986
1983	TH1 *T. ferrooxidans*, 50-56% organic sulphur removal from lignite	pH = 3 50°C	Gokcay and Yurteri, 1983
1987	*Sulfolobus acidocaldarius* thioxanthene and thianthrene	pH = 3 70°C	Kargi, 1987
1985	*Pseudomonas* mutant, CB1, DBT, sulphide, thioanisole, 1-benzothiophene, n-octyl sulphide benzylmethyl, 18-47% organic sulphur removal from coal	pH = 7 25-40°C	Isbister and Doyle, 1985 Isbister and Kobylinski, 1985
1987	*Acinetobacter*, CB2, diphenylsulphide, benzylphenyl sulphide, 30% organic sulphur removal from coal	pH = 7 25-40°C	Couch, 1987
1990	CB3 DBT 90% reduction	pH = 7 25-35°C	Harding *et al.* 1990
1988	*Alcaligenes denitrificans* and *Brevibacterium* (79.5-91.8)% reduction of DBT	pH= 7.4 30°C	Van Afferden *et al.* 1988, 1990
1988	*Escherichia coli* strain Benzene sulphonic acid, cystine, thiophene. 0-7% total sulphur reduction of coal	pH = 7	Klubek *et al* (1988)
1987 - 1990	IGTS7, IGTS8, and IGTS9 *Rhodococcus rodochrous* and *Bacillus sphaericus* DBT,thianthrene, thioxanthene, 91% org. sulphur removal from coal	pH =7 30°C	Kilbane and Maka, 1987 Kilbane 1989 Kilbane and Bielaga, 1990a, b

1.3. FACTORS AFFECTING ORGANIC SULPHUR REMOVAL BY MICROBES.

The potential of microbial desulphurisation as a mean of reducing sulphur dioxide emission from coal fired installations has been studied by various laboratories in many different countries. Coal has both organic and pyritic forms of sulphur. Pyritic sulphur can be effectively removed from coal before combustion by physical, chemical and microbial means, however, the removal of organic sulphur is still problematical.
Developing a process that removes the organic sulphur content is a most difficult task. Several bacterial cultures have been claimed useful for removing organic sulphur (Roberto *et al.* 1991). The bacteria degrading organic sulphur compounds are most commonly heterotrophic and soil bacteria from the genera *Pseudomonas* and *Rhodococcus*. These bacteria require conditions near neutrality and are entirely different from the bacteria of the genus *Thiobacillus* which have the ability to remove pyritic sulphur from coal. *Thiobacillus ferrooxidans* does not remove organic sulphur from coal. Based on available microorganisms, a bioprocess that is intended to remove both types of sulphur in coal would need entirely different conditions or sequential reactors with a change of pH and growth medium. An attempt to develop an acidic process where the organic forms of sulphur would be attacked will require isolation and/or development of naturally occurring acidophilic strains possessing this capability.
In this part, an overview of supporting factors together with some problems/limitations of bio-organic sulphur removal from coal will be discussed.

1.3.1 SUPPORTING FACTORS

Possibility of finding microorganisms capable of degrading organic sulphur in coal

The study of Wichlacz and Unz (1981) demonstrated that there were a variety of acidophilic heterotrophs from coal mine drainage awaiting description. Populations of acidophilic, heterotrophic bacteria were found associated with *Thiobacillus* species. These species seemed able to metabolize simple organic sulphur moieties and thus could degrade a

small percentage of the organic sulphur remaining in the coal matrix (Kargi, 1982). Johnson *et al.* (1979) had also observed persistent growth in mineral salts of heterotrophs isolated as a contaminant from *Thiobacillus ferrooxidans* cultures and also isolated in acid streamers from a pyrite mine in Wales. They concluded that these acidophilic cultures grew on organic impurities in the growth medium and absorbed CO_2 from the atmosphere. Moreover, there were a variety of heterotrophic bacteria which could use the low concentrations of organic compounds available (including several compounds of organic sulphur) (Harrison, 1984). These organic compounds were probably produced and excreted by the autotrophic sulphidic-mineral oxidizing bacteria. These organisms had not received much attention and it is now apparent that the interaction of heterotrophic and autotrophic microbial populations is important. The contaminant (the heterotroph) was in association with the autotroph in the natural habitat and its role there awaited definition. The study of Radway *et al.* (1987) showed there was a substantial number of the aerobic heterotrophic microorganisms associated with coal. They suggested that these microorganisms might be capable of degrading the organo-sulphur component in coal. However, nothing was known of their development in fuel grade coal. The ability of selected isolates to degrade organo-sulphur compounds, e.g. thiophene is being investigated.

An acidophilic process to remove organic sulphur from coal.

The isolation of "new" microbes which could attack both forms of sulphur under the same conditions will be very much welcomed and will enhance the development of microbial coal desulphurisation. So far, organisms that attack pyritic sulphur at neutral pH have not been identified (Roberto *et al.* 1991). It is reasonable to attempt to develop an acidic process where the organic forms of sulphur would be attacked. This requires isolation and/or development of naturally occurring acidophilic strains possessing this capability, followed possibly by use of genetic engineering techniques to enhance their capabilities. The other advantage would be that the new microorganisms should be able to coexist with the *Thiobacillus* strain currently used in pyritic sulphur removal without too much interference and change to the environment of either.

Ability of microorganisms to adapt

The microorganisms should be capable of surviving and growing in a coal water slurry. Different salts are leached from coal. Some of these leached salts which may be toxic to microbes may precipitate as nutrients to the coal water slurry. Trace elements and organic compounds leached from coal may affect the growth of the microorganisms in different ways. An example of the different effect of toxicity of heavy metals was demonstrated by Olsson *et al.* (1989) who worked with *Sulfolobus* species, finding that *Sulfolobus acidocaldarius* was affected more by leached compounds from coal than were *Sulfolobus brierleyi* and *Sulfolobus solfataricus* . However, leached compounds do not necessarily affect the microorganisms in a negative way; it is of course possible that the leached salts are useful for growth and can act as an extra source of nutrition for the microorganisms. Different microorganisms react differently; some are inhibited but some flourish. Although initially the microorganism is inhibited (but not killed), it can adapt over a period of time and tolerate much higher concentrations of certain metals, cations, and specific compounds. Tolerance is obtained by a slowly increasing the concentration of the specific component(s) under study. The microorganisms of the culture will adapt themselves to the higher concentration of specific metals or compounds which are introduced slowly. Srivastava *et al.* (1989) mentioned that the ability of microorganisms to adapt to new environments was found both in pure cultures and in microbial consortia; in the latter, not only can the characteristic of each component organism change but the compositional balance within the consortium can also change dramatically.

Sulphur requirement

All microorganisms require sulphur for growth. This requirement is the basis of a selection technique for isolating specific microorganisms. In a bioreactor, all the conditions and nutrients (required for the healthy organism growth) except sulphur are supplied. Coal with a certain content of organic sulphur with no or very low pyritic sulphur is present in the reactor. Therefore, microorganisms either survive by utilizing sulphur from the coal, or die if they fail to do so. The evolution of a culture with desulphurising ability is accelerated by chemical

mutagenesis. This technique was used by Kilbane to isolate IGTS8 microorganisms which were claimed to be capable of removing organic sulphur from coal (Kilbane, 1989).

1.3.2. PROBLEMS AND LIMITATIONS

Despite its potential, the microbial desulphurisation of coal has its problems and limitations. Basically coal is not an ideal material for biological attack, because of the chemical structure and the physical properties of coal. In the following, some problems which limit microbial activity will be discussed.

Surface availability

Coal is an organic rock of high density and its molecules can not pass cell membranes. Another potential problem related to coal is the surface availability of the organic sulphur for microbial attack. Biological reactions are limited to the external surface of the coal particle and possibly only within its macro- and mesopores. This part of the external surface is relatively small compared with the total surface (including micropores). Organic sulphur is bound chemically within the coal structure and is an integral part of coal's molecular matrix. It is not readily accessible to microbial attack. The surface availability of the organic sulphur is limited for microbial attack, so its breakdown is another problem facing the microbes.

Heterogeneity of coal

Chemically, coal is heterogeneous. Even within the same coal sample the aromatic structures of coal vary in size as well as in the type and number of substituents. The different linkages of the organic sulphur in coal cannot be determined separately. Therefore, no specific sulphur compounds are shown to serve as the substrate of a microbial attack. Because of the heterogeneity of coal many researchers preferred to use DBT instead of coal, although some still doubt whether the microbes which work well with DBT would produce the same result when working with different types of coal. This means that the biological removal of organic sulphur from coal may have to be tailored separately

to each coal. Use of defined sulphur compounds as sole sulphur sources allows an understanding of the utilization of these compounds by particular microbes. In the case of coal which contains a variety of organic and inorganic sulphur compounds it is not an easy task to study the selective utilization of each sulphur source.

Chemical analyses of sulphur in coal

In addition to the problems associated with the removal of organic sulphur from coal, the direct measurement of organic sulphur in coal presents difficulties. The different linkages of the organic sulphur in coal cannot be determined and this has been an important factor in the controversy over the effectiveness of organic sulphur removal from coal by microbiological methods (Olson *et al.* 1986).

Organic sulphur is calculated in ASTM method D 2492-84 as the difference between total sulphur and the sum of pyritic and sulphate sulphur. Lack of direct assay for organic sulphur determination exacerbates the difficulty of efforts to make a clear connection between microbial metabolism and modification of the coal matrix. Thus, the development of a method to analyze the different types of organic sulphur in coal will be needed in the future. In order to avoid errors, researchers have tried to determine organic sulphur directly. Already, some research in determining organic sulphur directly has been done in several laboratories. Methods developed include:

1. Electron probe microanalysis (Sutherland, 1975).
2. Scanning electron microscopy with energy dispersive X-ray analysis, SEM-EDAX (Straszheim *et al.* 1983).
3. Transmission electron microscopy (TEM) (Hsieh and Wert, 1985).

The direct determination of organic sulphur is not easy and the methods available are complex and expensive.

There is great need for the development of a rapid, inexpensive, accurate and precise alternatives.

Note: in this study the uncertainties and problems of chemical analyses of sulphur content will not be discussed.

All the coal samples were analyzed by Coal Research Association of New Zealand, Lower Hutt.

Stability of microorganisms

The results of previous studies for organic sulphur removal showed that there was a lack of stability of desulphurisation abilities in microbial cultures (Cough, 1987). Possible instability of genetically engineered microbial cultures is another problem. Over extended periods of usage, an originally effective desulphurisation organism may give rise to spontaneous derivatives that lack desulphurisation ability. Nearly every researcher who has reported the isolation of a bacterial culture capable of metabolizing organo-sulphur compounds (DBT) has subsequently found or reported that this trait was so unstable that the active cultures were lost completely and could not be reisolated (Kilbane and Bielaga, 1990b). In this case, the need for bacterial cultures that are stable is an important factor to encourage further study.

Long period of incubation time and complete sulphur removal

The long residence time required in bioprocessing is a major obstacle to the usefulness of this technology. Low biodesulphurisation rate, slow bacterial growth and low process yield may all contribute to limit future application of biodesulphurisation There is also a need for complete sulphur removal from coal. So far only pyritic sulphur can be removed effectively by using microorganisms. On the other hand, almost all microorganisms capable of removing organic sulphur in model compounds have been recovered from microbial populations isolated from soil near coal mines or petroleum refineries. These microorganisms are aerobic bacteria that remain active only in a neutral or alkaline medium at temperature between 25° and 35°C. They are unable to coexist with the acidophilic bacteria which are necessary for pyritic sulphur removal. There is a need to identify new acidophilic organisms that have broad organic sulphur removal capabilities and that can coexist with other bacterial strains currently used for pyritic sulphur removal such as *Thiobacillus ferrooxidans*.

1.4. COAL STRUCTURE AND FORMS OF ORGANIC SULPHUR IN COAL.

Coal is fossilized plant material formed from the remains of plants that flourished millions of years ago. It is carbonaceous, nonhomogeneous contains many constituents of its ancient swampy environment such as clay minerals, dissolved salts and sulphur. Under the influence of heat, pressure and geologic time, the plant fragments are altered, minerals are transformed, and volatile components removed.
The majority of sulphur present in coal is either combined to form metal sulphides, mostly iron sulphides (pyritic sulphur), or is covalently bound to the hydrocarbon compounds forming the coal matrix (organic sulphur). Pyritic sulphur has a well defined chemical and mineralogical composition and is physically separated from the coal matrix. By contrast the organic sulphur is integrated into the macromolecular coal matrix in a reduced form such as thiol, sulphide, disulphide, and thiophene and hence it can be regarded as a structural element of the coal. Very little is known about the exact nature of organic sulphur in coal because satisfactory analytical methods are lacking.

1.4.1. STRUCTURE OF COAL

The true structure of coal is at present unknown. Most workers agree that the structure is so complicated and variable that is impossible at this time to form an accurate model of it. However, despite the present inadequate knowledge of coal constituents, many attempts have been made to study the structure of coal and through these attempts the knowledge of its general character has been greatly improved.

Coal model structure

Coal is a complex polymeric solid with no repeating monomeric units. Coal is insoluble in most common organic solvents and is composed of variously substituted condensed polynuclear systems which consist of aromatics and hydroaromatics. Specifically, the basic structure of coal is a graphite-like aromatic/hydroaromatic system (Gavalas, 1982).

The diagram shown in figure 1. 5. is an attempt by Wiser (1973) to use the present knowledge of coal to represent an average bituminous coal in a single plane, showing the aromatic and hydroaromatic components.

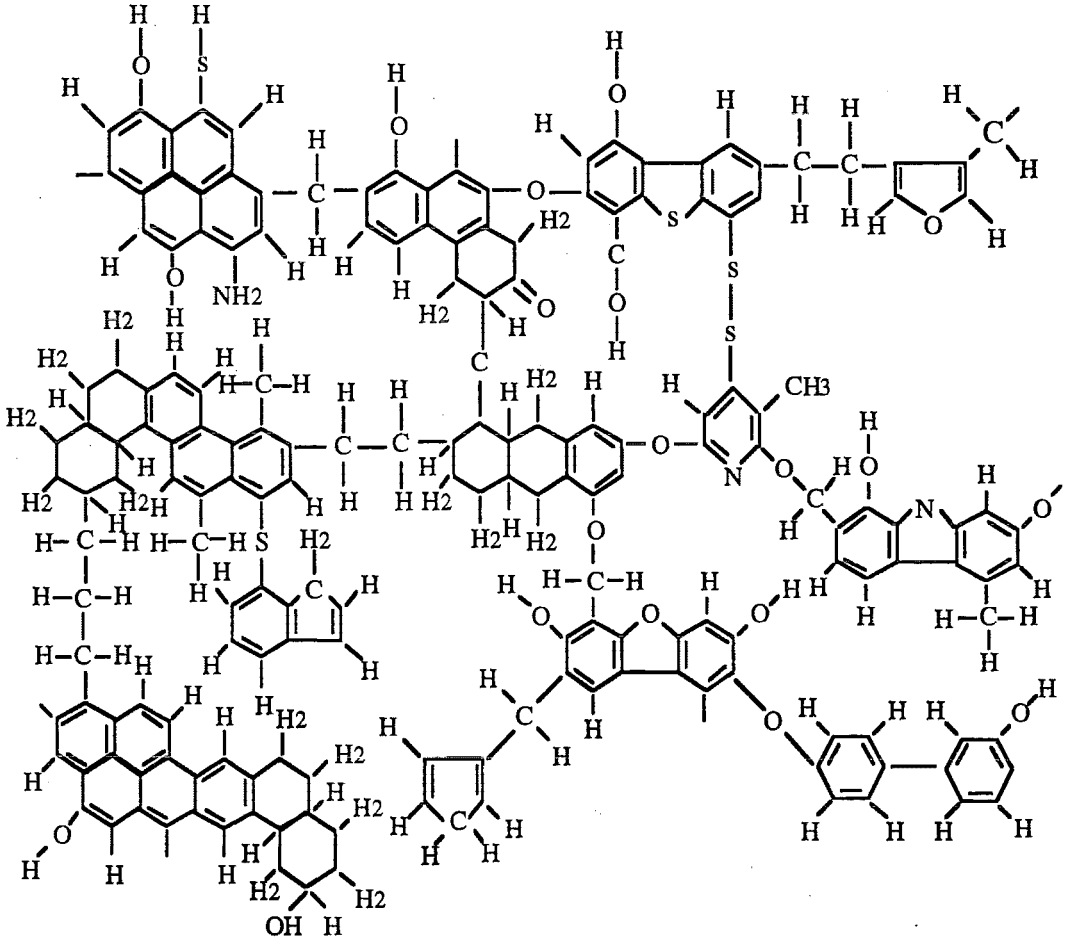


Figure 1. 5 : Suggested structure of bituminous coal (From Wiser, 1973).

Wiser's model modified the extensive hydroaromatic structures by introducing relatively weak bonds linking the multi ring aromatic units of coal. The layers may then consist of several aromatic clusters, each perhaps randomly oriented in a plane different from the surrounding clusters. This is a theoretical model, but it is representative of the current attempts to improve the understanding of coal structure. Wiser's diagram does not rule out the possibility of three dimensional molecules in coal. Given (1960) and Gibson (1978) have also proposed another model molecule structure for bituminous coal. Despite significant differences amongst these proposed structures they all contain relatively small condensed aromatic systems, consisting, on average, of two or four

condensed rings: phenanthrene and fluorene-type condensed aromatic rings predominate; the nonaromatic part of the molecule consists mostly of cyclo-paraffinic and hydroaromatic rings systems; and there are few alkyl (mainly methyl) groups. In coal, aromatic constituents are crosslinked either by covalent bonds or by hydrogen bridge bonds which make them largely resistant to solvents. This crosslinked structure is the main reason for the low extractability of coal components and subsequent difficulties in its chemical treatment.

1.4.2. ORGANIC SULPHUR IN COAL

According to Van Krevelen (1961) sulphur in coal may occur in three forms as follows:
1. the mineral pyrite or marcasite (commonly known as inorganic sulphur)
2. sulphate
3. organic sulphur combined with the coal material .
The inorganic/pyritic and organic sulphur account for almost all the sulphur in coals. Sulphate sulphur is usually much less than 0.1 % in freshly mined coals. Although sulphur is usually encountered in the three forms mentioned above, elemental sulphur concentrations as high as 15% have been found by some workers.
The organic sulphur in coal has two origins: the original plant sulphur and resulting from the incorporation of elemental sulphur and hydrogen sulphide sulphur in subsequent geochemical stages. Elemental sulphur probably arises either from the oxidation of hydrogen sulphide in contact with oxygen dissolved in interstitial waters or from microbial activity. Organic sulphur compounds may have formed during an early stage of the coalification process (humification) when plant debris is decomposed by bacterial activity to premaceral humic substances. The source of the organic sulphur in coals is usually considered to be the sulphur-containing amino acid structures that are found in both plant and animal proteins (Casagrande *et al* . 1977).

The organic sulphur and oxygen in the hydrocarbon matrix of coal (coal substance) are exchangeable to some degree. However their presence in coal affects the coal characteristics in different ways (Pauling, 1945). Organic sulphur is usually distributed throughout the coal and, cannot be

removed by conventional physical coal cleaning methods. The organic sulphur is part of and linked into the coal itself; it cannot be removed unless the chemical bonds holding it are broken. Only a far-reaching chemical degradation can release these sulphur atoms.

Organic sulphur groups:

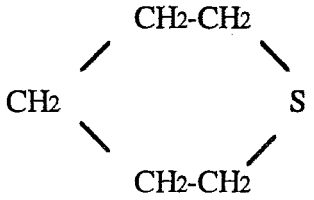
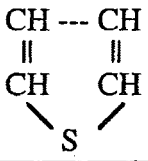
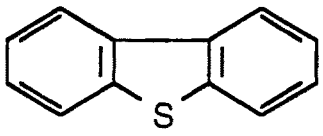
Little is known about the chemical structure of organic sulphur compounds in coal. At best, current knowledge is qualitative and is based almost entirely upon characterization of volatile products.
Organic sulphur in coal is categorized according to the type of functional group in which it appears. According to Given and Wyss (1961) there are five functional groups of organic sulphur:
1. mercaptan or thiol, R-SH
2. thio-ether or sulphide, R-S-R'
3. disulphide, RS-S-R'
4. aromatic systems containing the thiophene rings
5. y-thiopyrone systems
where R and R' designate alkyl or aryl groups.
Thiol and disulphide are likely secondary products because they are thermally rather unstable and would not survive the coalification process. The thiophenic compounds are found in coal hydrogenation products. The characteristic of the organic sulphur groups is presented in appendix A.

Types of organic sulphur in coal:

The exact forms of organic sulphur compounds present in coal are not known but major groups have been classified. One example of the types of organic sulphur in coal can be seen in table 1. 2. (Kargi, 1982).
In 1952, Kreulen stated that a large part of the organic sulphur in Istria coal was present in ring structures, and that sulphur humic acids containing 8% sulphur were obtained by oxidizing the coal.
The majority of the organic sulphur in coal is believed to be in the form of aromatic sulphur, such as thiophenes, benzothiophenes, dibenzothiophenes (DBT), and thioxanthenes; this type of sulphur cannot be easily determined by present analytical procedures (Couch, 1987).

Table 1.2.

The main groups of organic sulphur compounds present in coal (Kargi, 1982)

Compound	Chemical composition
ethanethiol	CH_3CH_2SH
2-propanethiol	$(CH_3)_2$-CHSH
benzenethiol	C_6H_5SH
diethyl sulphide	$CH_3CH_2S\ CH_2CH_3$
thiocyclohexane	
diethyl disulphide	$CH_3CH_2SSCH_2CH_3$
dimethyl disulphide	CH_3SSCH_3
thiophene	
dibenzothiophene	

Hayatsu *et al.* (1978) determined the relative abundance of aromatic and heteroaromatic compounds in three coals of different rank (lignite, bituminous coal and anthracite) and found that the sulphur containing aromatic compounds such as benzothiophene, dibenzothiophene and benzonapthothiophene, were present in bituminous coal and anthracite, but not in lignite. Thus, the abundance of various types of organic sulphur compounds in coal may be related to the rank of the coal.

Attar (1979) determined the order of difficulty of removal of this type of organic sulphur and found that dibenzothiophene was the most difficult followed by thiophene, benzothiophene and napthothiophene.

In general, the fraction of sulphur present in each of the functional groups is not known with certainly but several estimates are available.

(i). Attar and Dupuis (1979) estimated that 10-30% of the organic sulphur in bituminous coals was present in the form of thiols.

(ii). Sulphidic sulphur accounted for 5-27% and thiophenes constituted 40-70% of the organic sulphur (Attar and Corcoran, 1977; Shah and Cronauer, 1979; Attar and Dupuis, 1979).

(iii). Most of the organic sulphur was thought to occur in single and condensed thiophenic rings (Shah and Cronauer, 1979).

(iv). In 1987, Boudou *et al.* reported that the organic sulphur in coal was integrated in the macromolecular coal matrix in the form of thiolic, sulphidic, and thiophenic substructures.

(v). A study of Kelemen *et al.* (1991) showed that some New Zealand coals consisted of (62-70)% of thiophene and (26-78)% of sulphidic sulphur.

(vi). An estimated 40-70% of the organic sulphur in American coals was present in the form of thiophenes and condensed thiophenic structures (Casagrande *et al*. 1977)

CHAPTER 2

ENRICHMENT AND IDENTIFICATION OF ACIDOPHILIC CULTURES

2.1. ENRICHMENT AND ISOLATION OF ACIDOPHILIC CULTURE

2.1.1. INTRODUCTION

One of the objectives of this study was to investigate the ability of isolated acidophilic cultures to remove organic sulphur from coal and which could coexist with acidophilic *Thiobacillus* strains currently used in pyritic sulphur removal. Initial research was directed towards setting up an enrichment (selection) of desulphurizing strains of microbes.
A sulphur limited batch reactor and enrichment technique similar to those used by Kilbane (1989) was used. Studies were based on the premise that all microorganisms require sulphur for growth, and that microorganisms survive by utilizing available sulphur sources.
Another important factor is the potential of all living entities including microorganisms, to adapt to changing environmental circumstances; not only do the characteristics of each component organism change, but the compositional balance within the consortium can also change dramatically (Srivastava *et al.* 1989).

2.1.2. EXPERIMENTAL

Reactor

Glass reactors were used in the adaptation and isolation stages.
The reactor has a working volume of 1.3 litre, equipped with an air flow line and stirrer. The reactors were set up in a controlled temperature water bath. There were six reactors; four reactors with medium H1 and H2 were inoculated with microorganisms; the remaining two were control reactors which were not inoculated.
The reactors were incubated for 180 days.
Conditions: air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.
Reactor configurations: see part I-chapter 2.

Culture media

The basic culture medium was sulphur free in its composition. Sulphur was added to the basic media in the form of coal (10%w/v), see later. There were two types of growth medium used in the experiment, medium H1 and H2. Their composition were as follows:

MEDIUM (H1)

NH_4Cl	0.8 gram
KH_2PO_4	0.4 gram
$MgNO_3.6H_2O$	0.2 gram
Distilled water	1.0 litre
Coal	10% w/v

MEDIUM (H2)

$MgCl_2.6H_2O$	0.2 gram
KNO_3	1.0 gram
$CaCl_2.2H_2O$	0.1 gram
$FeCl_3.6H_2O$	0.01 gram
K_2HPO_4	2.0 gram
Distilled water	1.0 litre
Coal	10% w/v

Note: distilled water were sterilized by autoclaving and medium H2 was slightly modified from the original medium described by Van Afferden, (1990).
The pH of the medium was adjusted to 3.0 with 2.0 M NaOH or 2.0 M HCl.

Microbial source

The effluent from earlier experiments investigating inorganic sulphur removal was the source of the inoculum (see part I-chapter 2). Each reactor was inoculated with 150 ml effluent. The mixed acidophilic culture previously used for pyritic sulphur removal was assumed to be a good source for other acidophilic microbes which have the potential to degrade organic sulphur compounds. This is supported by other researchers, such as Wichlacz and Unz (1982); Kargi (1982); and Harrison (1984).

Coal

The coal used was pretreated Benneydale coal which had no or very little pyritic sulphur due to its treatment with *Thiobacillus* mixed culture (see part I-chapter 3.2.1). Before the test, the coal was washed with hydrochloric acid to remove any sulphate sulphur present. Coal particle size was less than 105 μm. Coal was dried in an oven at 40°C for 24 hours before and after the experiment .

Analytical method

Microbial action was detected by comparing the total sulphur content (i.e. organic, sulphate and pyritic sulphur) in coal samples before and after microbial action.
Total, sulphate and pyritic sulphur were determined gravimetrically. Organic sulphur was calculated indirectly by subtracting the sulphate and the pyritic sulphur content from the total sulphur content. The sulphur analyses were conducted by the Coal Research Association of New Zealand, Lower Hutt, New Zealand.

Table 2.1.
Sulphur assay before and after the first stage of isolation

No Exp.	Medium	Sulphur assay (%) Before test				Sulphur assay (%) After test				Incubation (days)	% Total S removal	% Organic S removal
		S_{tot}	S_{Sulp}	S_{pyr}	S_{org}	S_{tot}	S_{Sulp}	S_{pyr}	S_{org}			
A1	H1	1.49	0.00	0.04	1.45	1.25	0.00	0.05	1.20*	180	16	17
A2	H2	1.49	0.00	0.04	1.45	1.46	0.00	0.05	1.41*	180	2	3
A3	H1	1.51	0.00	0.03	1.48	1.39	0.00	0.13	1.26*	180	8	15
A4	H2	1.51	0.00	0.03	1.48	1.45	0.00	0.05	1.40*	180	4	5
A5(**)	H1	1.48	0.00	0.08	1.40	1.48	0.00	0.13	1.35*	180	0	4
A6(**)	H2	1.48	0.00	0.08	1.40	1.48	0.00	0.11	1.37*	180	0	2

S_{tot} = total sulphur
S_{pyr} = pyritic sulphur
S_{sulp} = sulphate sulphur
S_{org} = organic sulphur
T. ferrooxidans and HCl treated Benneydale coal, size < 105 µm.
Conditions: air flow rate: 1.5 litre/minute; temperature: 37° C; stirrer speed: 125 rpm.
(*) Coal samples treated with HCl before chemical analyses
(**) Controls, not inoculated

2.1.3. RESULTS AND DISCUSSION

The results of the isolation work are presented in table 2.1.
This table summarizes the results in growth media, H1 and H2, after 180 days of incubation. The results of the experiments (A1-A6) showed there was some reduction of organic and total sulphur content of the coal samples. The percentage of organic sulphur reduction in the inoculated reactors (A1, A2, A3 and A4) was within the range 3-17%.
These results also showed that there was a reduction in the total sulphur of the coal samples which was within the range of 2-16%. No reduction in total sulphur occurred in control (not inoculated) reactors although organic sulphur was reduced by 4% and 2% in reactors A5 and A6.

The amount of organic sulphur reduction in reactors with medium H1 was higher than that from reactors with medium H2. Reactors with medium H1 (A1 and A3) showed 15% and 17% of organic sulphur reduction compared with 3% and 5% in the reactors A2 and A4 with medium H2. The total sulphur reduction was 8% and 16% in reactors A1 and A3, compared with 2% and 4% in reactors A2 and A4.

At present, while it is too early to determine the full potential of these enriched cultures in medium H1 and medium H2, designated HB1 and HB2, respectively, this preliminary investigation demonstrated that the mixed acidophilic cultures in media H1 and H2 have a definite capacity to remove organic sulphur from coal.
These data also showed that the performance of HB1 (in terms of organic and total sulphur removal) was different from that of HB2. This was probably due to the different types of microorganisms that developed in each reactor. However, further investigation is needed to examine this difference and it is discussed at a later stage (chapter 3. 4). The mixed cultures, HB1 and HB2, may contain a host of promising acidophilic microbes that are capable of degrading organic sulphur and probably of degrading aromatic compounds. Further studies to examine and to characterize these enriched cultures are necessary.

2.1.4. CONCLUSION

These preliminary investigations indicated that the microorganisms enriched in these experiments (cultures HB1 and HB2) had the capacity to remove some of the organic sulphur content from the coal. The long acclimatisation i.e. 180 days might have produced a desirable change in the microorganism's capability. There is good reason to suppose that these microorganisms collectively have the capacity to break down organic sulphur in coal.
These findings also suggest the possibility of complete sulphur removal from coal although further studies are needed in order to obtain more information about the microorganisms themselves, their optimum conditions, growth media, pathways etc.

2.2. IDENTIFICATION OF THE ACIDOPHILIC CULTURE

A preliminary investigation of the heterotrophs within the mixed acidophilic cultures from two batch reactors with different compositions of the media, H1 and H2, has been conducted; (Annink, M. 1991).

Preliminary results showed that the isolated cultures were a consortium of gram positive, spore forming, cocci and rod shaped bacteria, together with several fungal species and some unidentified organisms (bacterial and fungal). The bacteria were characterized as belonging to a motile *Bacillus* species with peritrichous flagella. Only one type of fungus was isolated from medium H1 and identified as an *Aspergillus* species. Two types of fungi were identified from the reactor with medium H2, a *Penicillium* species and an *Aspergillus* species

The *Penicillium* but not the *Aspergillus* species seemed to be able to solubilise the coal substrate, which provided an encouraging prospect for converting solid coal to a more readily useable fuel in liquid form and at the same time reducing its sulphur content. These results provide an exciting prospect for further research. Further experiments on the *Penicillium* species could confirm that the coal is being solubilised.
Some literature (Cohen and Gabriele, 1982; Ward, 1985; Catcheside, 1990) have shown that a range of wood degrading microorganisms can solubilise lignite and brown coal. These include members of the fungal

genera *Aspergillus, Candida, Paecilomyces, Penicillium* , unidentified *actinomycetes* and bacteria. The microbial solubilisation of coal has been studied with several fungi, both surface and submerged cultures (Scott and Strandberg, 1985). Various types of coal and the use of pretreatment have been evaluated. It has been demonstrated that some types of coal can be solubilised by microbial action, although the process and the products are not clearly understood.
The results obtained to date are intriguing, but additional research is required (Scott *et al.* 1986).

The isolated acidophiles, nevertheless, may represent a surprising variety of biotypes and it will be of interest to look at the other microorganisms present in the reactors. There are numerous strains of related species in the acidophilic environment waiting to be isolated and described.

CHAPTER 3

CHARACTERIZATION OF ISOLATED, MIXED ACIDOPHILIC CULTURES

3.1. INTRODUCTION

The result of the isolation experiments (see chapter 2) indicated that the mixed microorganisms, HB1 and HB2, degraded organically bound sulphur in coal. A major obstacle in this work was the long residence time (180 days) needed by the culture to degrade the organic sulphur from coal.

This chapter describes another set of experiments conducted with the following objectives:

(1). to examine the possibility that, HB1 and HB2, could degrade the organic sulphur in a shorter time, e.g. 30 days instead of 180 days.

(2). to examine how different physicochemical conditions affected the degrading activity of HB1 and HB2.

The principal physicochemical factors which influence the rate of degradation reactions and the level of microbial activities are temperature, pH, oxidation-reduction potential (Eh), together with other factors such as particle size and surface area of the coal substrate together with the concentration of nutrients and reactants (Ralph, 1985). Only the effect of particle size, temperature and coal type on the rate of organic sulphur removal has been investigated thus for.

The effect of coal particle size on the rate of organic sulphur removal was determined by using a pretreated Benneydale coal ground to three different particle sizes i.e. < 152 μm , < 105 μm and < 63 μm.
To investigate the effect of coal type on the rate of sulphur removal three different types of coal were used; pretreated Benneydale coal, "fresh" Benneydale coal and New Creek coal. The ability of the enriched cultures HB1 and HB2 to remove sulphur was examined with pretreated Benneydale coal and "fresh" Benneydale coal.
Their capacity to remove organic sulphur from New Creek coal (Buller area, West Coast of the South Island, New Zealand) which contains high organic sulphur (about 5%) was also examined.
The temperature of the medium is another important factor affecting the desulphurisation rate. Several experiments involving temperatures within the range 32° to 52°C have been conducted in an effort to ascertain the most suitable working temperature for these acidophilic cultures.
Organic sulphur content in coal samples was calculated as the difference between total sulphur and the sum of pyritic plus sulphate sulphur.

3.2. EXPERIMENTS WITH PRETREATED BENNEYDALE COAL

3.2.1. EXPERIMENTAL

Inoculum

From earlier results (table 2.1) only enriched cultures containing microbes with the capacity to degrade organic sulphur were selected. Inoculum (150 mls) from experiments A1 and A4 were used in the experiments described. The inoculum from reactor A1 was used to inoculate the reactor containing medium H1 and that of reactor A4 was used to inoculate the reactor containing medium H2 .

Experimental conditions

The condition of the reactors was similar to that previously described (chapter 2.1.2). The reactor conditions were as follows:

air flow rate: 1.5 litre per minute,

temperature: 37°C,
stirrer speed: 125 revolution per minute (rpm) and
10% w/v of coal was used as the substrate in media H1 and H2.

The experiment was conducted for 30 days.
Initial pH of the medium in each reactor was adjusted to the desired pH (3.0) with 2.0 M HCl or 2.0 M NaOH.

Coal:

Coal used in the experiments was pretreated Benneydale coal ground to particle size < 63 μm. This coal had been treated with *Thiobacillus ferrooxidans* to remove its pyritic sulphur content and washed throughly with acid to remove sulphate.

Analytical method

Total sulphur and forms of sulphur in coal (sulphatic, pyritic sulphur) were determined by CRA of New Zealand in Lower Hutt. The organic sulphur was calculated as the difference between the total sulphur and the sum of sulphatic and pyritic sulphur.
The change of pH medium in the reactor was detected by a pH-meter.

3.2.2. RESULTS AND DISCUSSION

The objective of the experiment was to confirm that the mixed cultures, HB1 and HB2, were capable of degrading the organic sulphur from coal in a relatively shorter incubation period. For this purpose, pretreated Benneydale coal ground to < 63 μm was used.
The results obtained in these first stage experiments after 30 days of incubation period are summarized in table 3.1.
Experiments using culture HB1 in medium H1 showed that the reduction of organic sulphur was within the range (14-15)%.
However, culture HB2 in medium H2 showed that the organic sulphur reduction was within the range (9-11)%. These results confirm that both cultures, HB1 and HB2, can degrade organic sulphur from coal. They show a consistency in terms of organic sulphur reduction and are capable of degrading approximately the same amount of organic sulphur from coal in 30 days as 180 days.

Table 3.1.

Sulphur assay before and after experiments with < 63 μm pretreated Benneydale coal

No Exp.	Culture	Sulphur assay (%) Before test				Sulphur assay (%) After test				Incubation (days)	% Tot S removal	% Org S removal	Rate Org.S Removal mg/l.day
		S_{tot}	S_{Sulp}	S_{pyr}	S_{org}	S_{tot}	S_{Sulp}	S_{pyr}	S_{org}				
B1	HB1	1.61	0.00	0.12	1.49	1.44	0.02	0.14	1.28	30	11	14	6.1
B2	HB1	1.35	0.00	0.05	1.30	1.31	0.13	0.06	1.12	30	3	14	5.3
B3	HB1	1.35	0.00	0.05	1.30	1.22	0.06	0.05	1.11	30	10	15	5.5
B4	HB1	1.35	0.00	0.05	1.30	1.29	0.11	0.06	1.12	30	4	14	5.6
B5	HB1	1.36	0.00	0.06	1.30	1.26	0.04	0.10	1.12	30	7	14	4.7
B6*	HB1	1.47	0.00	0.08	1.39	1.45	0.02	0.07	1.36	60	1	2	0.4
C1	HB2	1.59	0.00	0.07	1.52	1.46	0.02	0.06	1.38	30	8	9	3.3
C2	HB2	1.60	0.00	0.07	1.53	1.45	0.02	0.08	1.36	30	9	11	4.9
C3	HB2	1.31	0.00	0.06	1.26	1.27	0.09	0.06	1.12	30	3	11	4.0
C4	HB2	1.59	0.00	0.07	1.52	1.45	0.02	0.07	1.36	30	9	11	3.9
C5	HB2	1.58	0.00	0.08	1.50	1.48	0.03	0.09	1.35	30	6	10	4.0
C6*	HB2	1.47	0.00	0.08	1.39	1.44	0.02	0.05	1.37	60	2	1	0.2

S_{tot} = total sulphur
S_{pyr} = pyritic sulphur
S_{sulp} = sulphate sulphur
S_{org} = organic sulphur

T.ferrooxidans and acid treated Benneydale coal, particle size < 63 μm.

Conditions: air flow rate: 1.5 litre/minute; temperature: 37° C; stirrer speed: 125 rpm

(*) Control sample - no microbial inoculation

The data in table 3.1. also show that sulphate sulphur was detected in coal samples after the test, although before the test there was no sulphate. The detection of the sulphate sulphur in coal samples after the test suggested that one of the end products of organic sulphur degradation employing the cultures, HB1 and HB2, was sulphate, probably in the form of sulphuric acid.

Other evidence which supported the fact that sulphate was one of the end products of this degradation was the pH of the medium. Table 3.2. shows the change of pH in medium during the process. Decreasing pH values indicated that there was an increase in H^+ concentration due to release of sulphuric acid into the medium as a result of organic sulphur degradation by microbial action. Increase of H^+ concentration in experiments B1 to B5 ranged from 12 % to 109% and in experiments C1 to C5 ranged from 35% to 129%. In contrast, pH in the control reactors (experiments B6 and C6) showed negligible change. Increase of H^+ concentration in control reactors B6 and C6 were 2% and 5%, respectively.

Sulphate release due to microbial attack was also shown by Kargi and Robinson (1984) and Isbister and Kobylinski (1985) although the biochemical mechanisms leading to a release of sulphate had not been investigated. Sulphate as a product in the transformation of organic sulphur in coal had also been examined in several studies of coal weathering under natural and artificial conditions. During weathering, it appeared that the postulated organic sulphur species in coal, such as thiols, sulphides, disulphides, and thiophenic residues probably formed sulphones, sulphonic acids and sulphates, depending upon the severity of conditions (Liotta *et al.* 1983, and Calemma *et al,* 1988).

The data also showed that the amount of total sulphur detected in coal samples after the test was lower than the amount determined before the test. This indicated that some sulphur had disappeared during the process. Some of this sulphur has been detected in the form of sulphate sulphur in solid coal samples, however, the sulphur calculation showed there was still some sulphur missing after incubation. This sulphur might have been converted to water soluble forms, although this has not been studied. This result gives good reason to suppose that this degradation of organic sulphur from coal by the acidophilic cultures, HB1 and HB2, resulted in end products in the forms of sulphate and perhaps

other water soluble products. However, this interpretation needs further investigation. These products probably include some microbial products as well as coal derived components. Cohen and Gabriele (1982) reported that biodegradation of a North American lignite resulted in complex high molecular weight water soluble products. These breakdown products have been shown to contain aromatic, carboxylic and aliphatic carbon. Moreover, a study conducted in 1988 by Barik *et al.* showed that a consortium of microorganisms was able to convert coal to organic acids, ethanol, methane and in principle to other chemical compounds and fuels.

3.2.3. CONCLUSION

The result confirms that the previous isolation experiment (chapter 2) has successfully isolated an acidophilic microorganism or a consortium of microorganisms that are capable of degrading and utilizing the organic sulphur from coal. These cultures, HB1 and HB2, degrade similar amount of organic sulphur from coal in 30 days as occurred in 180 days. These indications are intriguing because:

Firstly: the enriched acidophilic microorganisms, HB1 and HB2, are capable of degrading organic sulphur from coal.

Secondly: the end products of the degradation are sulphate/sulphuric acid and some water soluble forms.

The results from the mixed cultures, HB1 and HB2, are encouraging and justify accelerated research into effective economic bioprocessing of coal.

Table 3.2.

pH changes for experiments B1-B6, C1-C6.

Day	*pH of experiment*											
	B1	B2	B3	B4	B5	B6	C1	C2	C3	C4	C5	C6
1	2.50	3.03	2.43	3.51	3.30	3.03	3.67	3.58	3.14	2.50	3.60	2.50
2	2.50	3.03	2.43	3.51	3.30	3.03	3.65	3.56	3.14	2.48	3.58	2.50
3	2.49	3.03	2.42	3.47	3.28	3.03	3.63	3.53	3.13	2.50	3.56	2.50
4	2.49	3.02	2.42	3.47	3.27	3.03	3.58	3.50	3.13	2.47	3.53	2.50
5	2.49	3.02	2.41	3.42	3.26	3.03	3.57	3.47	3.13	2.47	3.52	2.50
6	2.48	3.02	2.41	3.41	3.25	3.03	3.52	3.44	3.13	2.47	3.48	2.50
7	2.48	3.01	2.41	3.38	3.25	3.03	3.51	3.41	3.12	2.47	3.46	2.50
8	2.48	3.01	2.40	3.37	3.24	3.03	3.50	3.41	3.11	2.46	3.44	2.50
9	2.48	3.01	2.40	3.36	3.24	3.03	3.49	3.41	3.10	2.46	3.42	2.50
10	2.47	3.00	2.39	3.35	3.24	3.03	3.47	3.39	3.09	2.45	3.41	2.50
11	2.47	3.00	2.39	3.33	3.23	3.03	3.47	3.38	3.09	2.45	3.40	2.50
12	2.46	2.98	2.39	3.32	3.23	3.03	3.46	3.38	3.07	2.44	3.38	2.50
13	2.46	2.98	2.38	3.30	3.22	3.03	3.45	3.37	3.07	2.43	3.36	2.50
14	2.46	2.96	2.38	3.29	3.21	3.03	3.44	3.36	3.05	2.42	3.35	2.50
15	2.46	2.96	2.38	3.28	3.21	3.03	3.42	3.36	3.05	2.42	3.33	2.50
16	2.45	2.93	2.37	3.26	3.21	3.02	3.41	3.35	3.03	2.41	3.32	2.50
17	2.45	2.93	2.37	3.23	3.20	3.02	3.40	3.34	3.03	2.41	3.31	2.50
18	2.45	2.91	2.37	3.22	3.19	3.02	3.39	3.33	3.02	2.40	3.31	2.48
19	2.45	2.91	2.37	3.22	3.19	3.02	3.39	3.33	3.00	2.40	3.29	2.48
20	2.43	2.91	2.36	3.21	3.19	3.02	3.39	3.31	2.98	2.39	3.28	2.48
21	2.43	2.90	2.36	3.21	3.18	3.02	3.38	3.31	2.98	2.39	3.27	2.48
22	2.43	2.90	2.36	3.20	3.17	3.02	3.38	3.31	2.96	2.38	3.26	2.48
23	2.41	2.90	2.36	3.20	3.16	3.02	3.37	3.31	2.95	2.38	3.26	2.48
24	2.41	2.89	2.35	3.20	3.15	3.02	3.37	3.30	2.93	2.38	3.25	2.48
25	2.40	2.89	2.35	3.20	3.13	3.02	3.37	3.30	2.92	2.38	3.25	2.48
26	2.40	2.89	2.35	3.19	3.12	3.02	3.37	3.30	2.91	2.37	3.24	2.48
27	2.40	2.89	2.34	3.19	3.10	3.02	3.36	3.29	2.91	2.37	3.24	2.48
28	2.39	2.88	2.34	3.19	3.10	3.02	3.36	3.29	2.90	2.37	3.24	2.48
29	2.39	2.88	2.34	3.19	3.08	3.02	3.36	3.29	2.90	2.37	3.24	2.48
30	2.39	2.88	2.34	3.19	3.08	3.02	3.36	3.29	2.90	2.37	3.24	2.48
%[H^+] gain	12	41	23	109	66	2	104	95	74	35	129	5

3.3. EFFECT OF PARTICLE SIZE ON THE DESULPHURISATION OF COAL

3.3.1. PARTICLE SIZE AND CONVERSION RATE

Because of its chemical structure and physical properties, coal does not appear to be an ideal material for biological attack. Coal molecules are not accessible to direct biological attack. Coal consists of microporous constituents, which are surrounded by cleats and fissures as transport channels, as shown in the schematic diagram, figure 3.1., which gives a qualitative model of the pore structure.

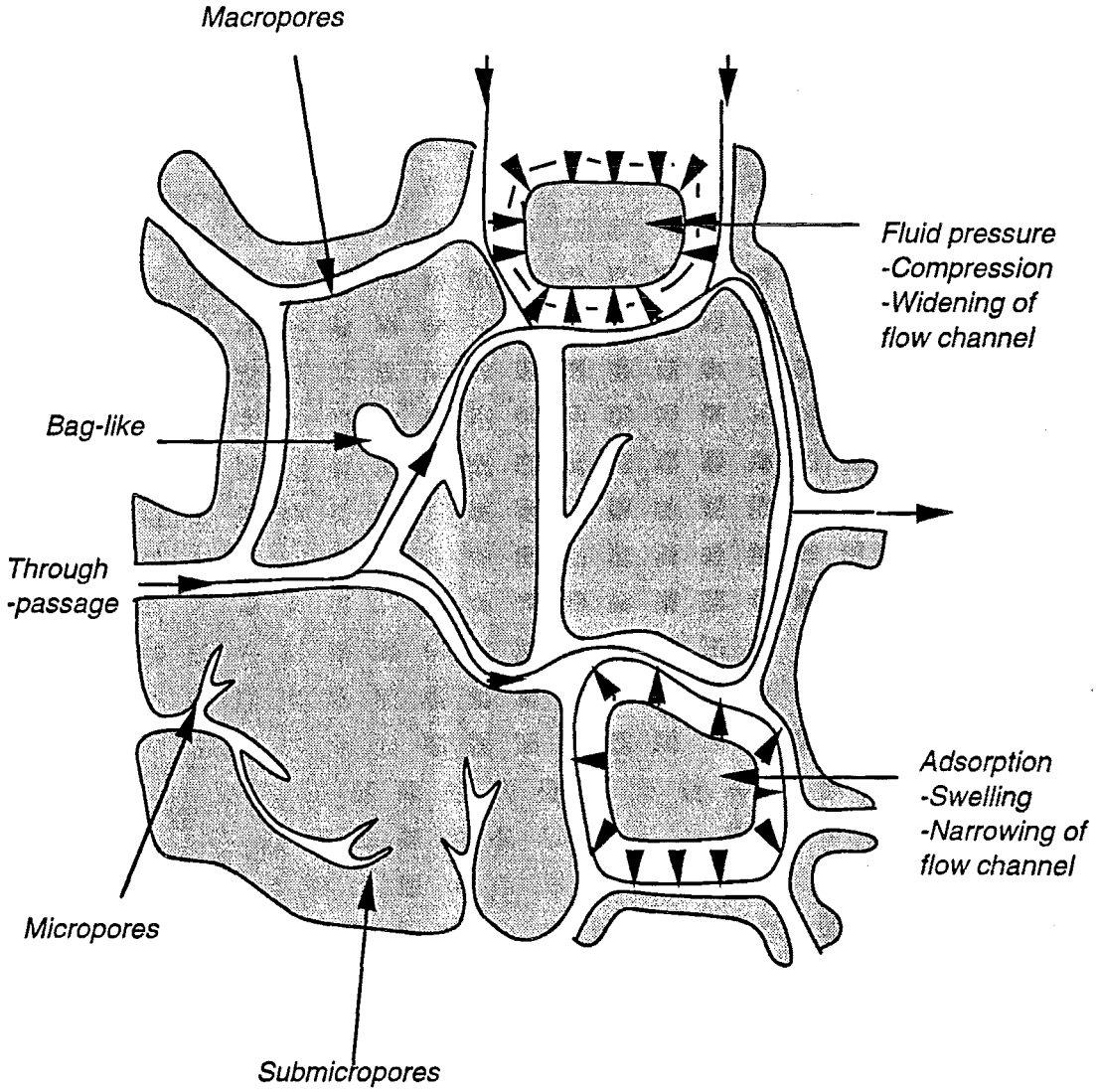


Figure 3. 1.: Pore system in coal (after Seewald *et al.* 1985)

Uhl *et al.* (1989) described the relation between the rate of conversion and the surface area. Their model assumed that the rate was a first order in surface area concentration and was presented by the following formula:

$$r_{s,j} = k_s S_j$$

where:

$r_{s,j}$ = the rate of conversion

k = a rate constant

S_j = surface area concentration

Parameter S_j will depend on the size of the coal particle.

This relation indicates that the efficiency of the rate of coal degradation is expected to increase as particle size decreases.

3.3.2. EXPERIMENTAL

Several experiments using pretreated Benneydale coal in batch reactors have been performed to determine the effect of particle size of coal on the rate of organic sulphur removal.

There were three different coal particle sizes used in these experiments:

- Dp < 152 μm (< 100 mesh BSS)
- Dp < 105 μm (< 150 mesh BSS) and
- Dp < 63 μm (< 240 mesh BSS),

where Dp was the diameter of coal particle size.

Typical particle size distribution range of each size is presented in appendix B.

The reactors were inoculated (150 mls) with the cultures, HB1 and HB2, derived from the previous experiment (page 34).

The initial pH of the medium in the reactor was adjusted to the desired pH (3.0) before the experiment with 2.0 M HCl and/or 2.0 M NaOH.

The reactors were incubated at 37°C with an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

The experiments lasted for 30 days.

3.3.3..RESULTS AND DISCUSSION

The result of these experiments with different particle sizes of coal substrate is presented in table 3.1, table 3.3 and table 3.4.

As expected, both HB1 and HB2 cultures showed increased organic sulphur degradation with decreasing particle size.
Table 3.1 (page 34) shows the result of the experiments with < 63 μm coal. The amount of organic sulphur removal were within the range of (14-15)% for culture HB1 and (9-11)% for culture HB2, after 30 days treatment .
In experiments with larger particle size < 105 μm, both the cultures, HB1 and HB2, appeared to be less effective in degrading organic sulphur, the amount of organic sulphur removal was found to be (8-9)% for culture HB1 and (7-8)% for culture HB2, after 30 days incubation.
In experiments with particle size < 152 μm the amount of organic sulphur removal were within the range of (6-7)% for culture HB1 and (3-5)% for culture HB2.
Control samples (unsterilized without inoculum) show negligible degradation (see experiments B6 and C6, table 3.1) with less than 2% of organic sulphur reduction after 30 days of incubation.

Figure 3.2 shows the comparison of the rate of organic sulphur removal from these experiments with three different coal particle size.

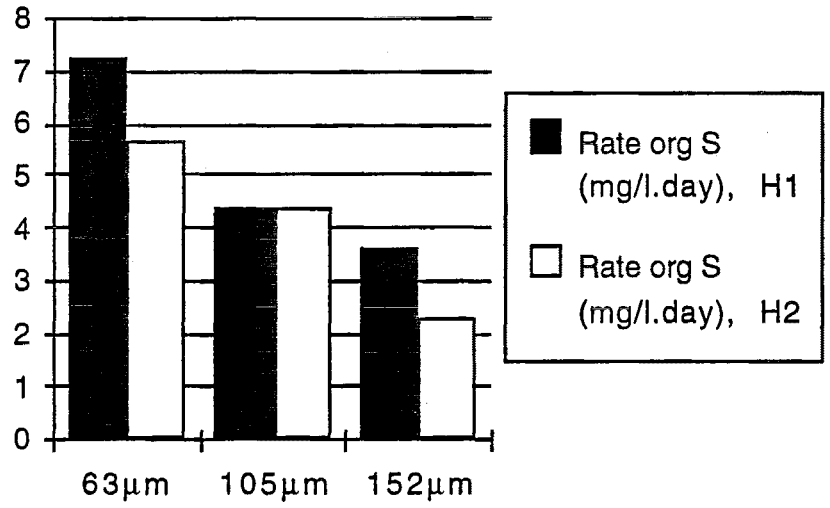


Figure 3. 2 : Rate of organic sulphur removal in relation to different particle sizes

Table 3.3.

Sulphur assay before and after experiments with < 105 µm pretreated Benneydale coal

No Exp.	Culture	Sulphur assay (%) Before test				Sulphur assay (%) After test				Incubation (days)	% Tot.S removal	% Org S removal	Rate Org.S Removal mg/l.day
		S_{tot}	S_{Sulp}	S_{pyr}	S_{org}	S_{tot}	S_{Sulp}	S_{pyr}	S_{org}				
D1	HB1	1.54	0.00	0.08	1.46	1.44	0.02	0.08	1.34	30	8	9	3.5
D2	HB1	1.33	0.00	0.06	1.29	1.28	0.05	0.05	1.18	30	4	9	3.0
D3	HB1	1.31	0.00	0.03	1.28	1.26	0.04	0.06	1.16	30	4	8	3.5
E1	HB2	1.29	0.00	0.06	1.30	1.26	0.02	0.08	1.16	30	2	7	4.4
E2	HB2	1.31	0.00	0.03	1.28	1.27	0.04	0.09	1.18	30	3	8	2.6
E3	HB2	1.58	0.00	0.09	1.49	1.50	0.03	0.09	1.38	30	5	7	2.9

S_{tot} = total sulphur
S_{pyr} = pyritic sulphur
S_{sulp} = sulphate sulphur
S_{org} = organic sulphur

T.ferrooxidans and acid treated Benneydale coal, particle size < 105µm.

Conditions: air flow rate: 1.5 litre/minute; temperature: 37° C; stirrer speed: 125 rpm

Table 3.4.

Sulphur assay before and after experiments with < 152 µm pretreated Benneydale coal

No Exp.	Culture	Sulphur assay (%) Before test				Sulphur assay (%) After test				Incubation (days)	% Tot S removal	% Org S removal	Rate Org.S Removal mg/l.day
		S_{tot}	S_{Sulp}	S_{pyr}	S_{org}	S_{tot}	S_{Sulp}	S_{pyr}	S_{org}				
F1	HB1	1.57	0.00	0.07	1.50	1.49	0.02	0.07	1.40	30	5	7	3.3
F2	HB1	1.31	0.00	0.03	1.28	1.23	0.01	0.05	1.17	30	6	7	2.5
F3	HB1	1.34	0.00	0.05	1.29	1.32	0.04	0.08	1.20	30	1	7	2.4
G1	HB2	1.52	0.00	0.05	1.47	1.48	0.02	0.06	1.39	30	4	5	2.3
G2	HB2	1.55	0.00	0.09	1.46	1.50	0.02	0.06	1.41	30	3	3	1.4
G3	HB2	1.51	0.00	0.05	1.46	1.48	0.02	0.05	1.40	30	2	4	1.4

S_{tot} = total sulphur
S_{pyr} = pyritic sulphur
S_{sulp} = sulphate sulphur
S_{org} = organic sulphur

T.ferrooxidans and acid treated Benneydale coal, particle size < 152 µm.

Conditions: air flow rate: 1.5 litre/minute; temperature: 37° C; stirrer speed: 125 rpm

From the data in table 3.1., table 3.3. and table 3.4., the mean rate of organic sulphur removal has been calculated and is shown in table 3.5.

Table 3.5.(*)

Mean rate of organic sulphur removal for pretreated Benneydale coal

Particle size (μm)	Rate of org.S removal ($mg.l^{-1}.day^{-1}$) HB1	Rate of org.S removal ($mg.l^{-1}.day^{-1}$) HB2
< 63	5.4	4.0
< 105	3.4	3.3
< 152	2.7	1.7

(*) Calculation based on data on table 3.1.; table 3.3.; and table 3.4..

This result shows that a reduction in coal particle size significantly improves the rate of organic sulphur removal. For culture HB1, a reduction in particle size from 152 μm to 105 μm resulted in an increase of the rate of organic sulphur removal by a factor of 1.2 and further reduction from 105 μm to 63 μm resulted in an increase by a factor of 1.6. For culture HB2, the factors are 1.9 and 1.2, respectively. The calculation shows that the reduction of particle size from 152 μm to 63 μm resulted in an increase of the rate of organic sulphur removal by a factor of 2.0 and 2.3, for culture HB1 and HB2, respectively.

If the increase in desulphurisation rate seen with the smaller particle is due only to the increased surface area of sulphur available for bacterial attack, then the rate should be proportional to the coal surface to volume ratio, i.e., inversely proportional to the particle size.
This was confirmed by plotting the rate against the reciprocal of the particle size (1/Dp), as in figure 3.3. This yielded best fit graphs with R^2 = 0.996 for culture HB1 and R^2 = 0.831 for culture HB2.
Particle size as an important factor in coal desulphurisation has been demonstrated by other researchers. For example, Dugan and Apel (1978) achieved their best result in an inorganic sulphur removal experiment using coal with particle size < 75 μm.

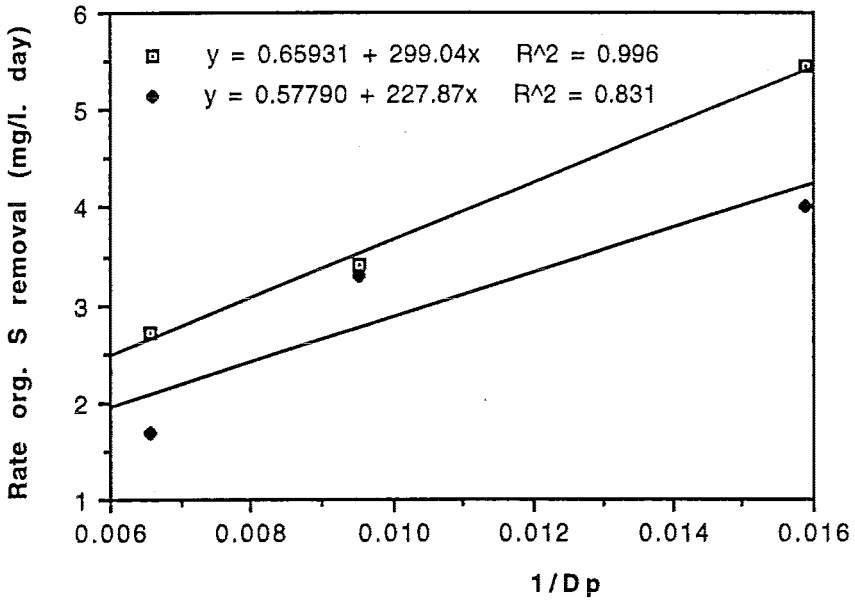


Figure 3. 3.: Rate of organic sulphur removal as a function of 1/Dp for pretreated Benneydale coal.

Andrew and Maczuga (1982) showed that good rates of desulphurisation were achieved with slightly smaller particle < 45 μm. Kargi and Robinson (1985) also reported that a reduction in particle size from 270 μm to 48 μm resulted in an increase in the rate of desulphurisation by a factor of 2.1.
They indicated that the rate of sulphur removal decreased hyperbolically with an increase in particle size and reached an almost constant level at a particle size of 250 μm. Further decrease of particle size to < 63 μm would probably lead to more organic sulphur removal , although in practice the optimum particle size has to be determined for each kind of coal to be leached.
Table 3.6. provides a general survey of the pore width distribution of hard coal and their accessibility by biological agents. For possible biological processes, accessibility of the coal surface for microorganisms or at least for their enzymes is required. Because of the porous structure of coal, biological reactions are limited and possibly only within its macro- and mesopores (d > 20 nm) (Klein *et al.* 1988). The micro/macropore systems as a whole should be regarded as flexible. The transport channels with diameters in microns are responsible for the permeability of coal. Here, gas and liquid flow takes place. Depending upon the nature of the deposited material and shear rate at particle surface, the particles may either expand or shrink despite the reduction in core size due to biological oxidation (Kargi, 1989).

Table 3.6:
Distribution of pore width in hard coal and their accessibility to microbes(*)

Types of pores	Diameter	Frequency	Accessible for
Fissures	> 5 µm	Tectonically influenced	fungi, bacteria,
Macropores	5 µm.- 50 nm	30%	enzymes
Mesopores	50 nm - 2 nm	10%	bacteria, enzymes
Micropores	2 nm - 0.8 nm	40%	enzymes (> 20 nm)
Submicropores	< 0.8 nm	20%	-

(*) Seewald *et al.* 1985.

Consequently, improvement in the rate of organic sulphur removal is possible if the organic sulphur is accessible to microbial attack. Surface area and pore size play a major role in the desulphurisation processes by affecting the accessibility of organic sulphur sites for microbial attack. The more finely ground the coal, the greater the surface area exposed to microbial action. The organic sulphur is integrated in the coal matrix and, in contrast to chemical oxidation, can only be attacked by direct contact between coal and biocatalyst. Hence, considerable desulphurisation should be expected for only the smallest particles. A reduction in particle size exposes more sulphur surfaces to microbial attack. It will result in substantial increases in the rate of sulphur leaching.

Because of its more complex chemical and physical structure (see also chapter I: coal structure), coal seems to be more resistant to microbial attack. However, it is well known that coals imbibe solvents and swell appreciably. Recent research into the macromolecular structure of coal, in terms of the molecular weight between cross links, could provide an important parameter in breaking down the bonds in the coal matrix in order to establish more access to the interior of a coal particle (Gorbaty, 1991). In this case, organic sulphur removal from coal may be best approached by a combination of chemical and biological steps. The chemical steps are directed towards modifying the sulphur bond and pore structure from the coal matrix to improve its biological accessibility.

However, to find a microorganism, or a consortium of microorganisms, which will break down particular bonds in ring structures, is not likely to be an easy task.

3.3.3. CONCLUSION

The ability of the mixed acidophilic cultures, HB1 and HB2, at removing organic sulphur from coal appears to depend on the particle size of coal substrate. Using pretreated Benneydale coal, the culture HB1 was more efficient in degrading organic sulphur than the culture HB2. In desulphurisation experiments with coal of particle size < 63 μm, up to 15% of organic sulphur was removed by HB1 and up to 11% by HB2 after 30 days of incubation. Decreasing particle size of coal from 152 μm to 63 μm resulted in an increase in the rate of organic sulphur removal by a factor of 2.0 and 2.3, for culture HB1 and HB2, respectively. Further decrease in particle size e.g.<63 μm would probably result in further increase in the organic sulphur removal rate, although the optimum size of coal particles would be dictated mainly by economics. Technically, by a combination of different grinding processes coal can be ground to a particle size of about 2.5 μm (Fakoussa, 1981 and Fakoussa and Truper, 1983), which provides suitable coal samples for the screening of potent microorganisms. However, such preparation needs high energy input and makes the industrial application uneconomic at present.

The work described here is preliminary, but further study into the effect of combining the chemical processes including particle size reduction and sulphur bond modification in the coal matrix with subsequent biological treatment should be pursued.

3.4. EXPERIMENTS WITH "FRESH" BENNEYDALE COAL

3.4.1. COMPLETE SULPHUR REMOVAL AND EFFECT OF TEMPERATURE

Back ground:

As mentioned in chapter 2 cultures HB1 and HB2 were isolated from an earlier pyritic sulphur removal experiment which employed a mixed culture with *Thiobacillus ferrooxidans* as the dominant bacteria. This raised the possibility that the mixed cultures HB1 and HB2 had the capability of degrading pyritic sulphur as well as organic sulphur. The ability of *Thiobacillus ferrooxidans* to remove pyritic sulphur is known, although *Thiobacillus ferrooxidans* is unable to remove organic sulphur as shown in this study and also by Dugan and Apel (1978); Andrews and Maczuga (1982); and Uhl *et al.* (1989). Only Gokcay and Yurteri (1983) revealed that a mesophilic THI strain of *Thiobacillus ferrooxidans* (at 50°C and pH 3.0) was able to remove 50% to 56% of organic sulphur and 90% to 95% of pyritic sulphur during 25 days incubation period of Turkish lignite.
In the light of the above, it is reasonable to suppose that the cultures HB1 and HB2 probably do have the ability to degrade pyritic sulphur along with their capacity at removing organic sulphur from coal as already shown in the result of this study (see chapter 3.2). To investigate this possibility experiments using "fresh" Benneydale coal were conducted.

Experimental:

The conditions of the experiments were the same as before:

air flow rate : .1.5 litre/minute;

stirrer speed: 125 rpm;

In the first series of experiments the temperature was set at 37°C.

Note: To understand the effect of temperature on sulphur removal rate, some experiments were conducted at different temperatures: 32°; 42°, 47° and 52°C.

Coal:

"Fresh" Benneydale coal which contains 2.61% total sulphur (1.08% pyritic and 1.53% organic sulphur) was used. It was ground to < 63 μm before the experiment. The sulphur content of the coal samples after the test was analyzed by CRA, Lower Hutt.

3.4.2. RESULTS AND DISCUSSION

Experiments at 37°C, air flow rate of 1.5 litre/minute, stirrer speed of 125 rpm.

As shown in table 3.7., the cultures HB1 and HB2 demonstrated that they could remove both types of sulphur (organic and pyritic sulphur) from "fresh" Benneydale coal samples.
Table 3.7. shows that culture HB1 removed up to 85% pyritic sulphur and up to 12% organic sulphur whereas culture HB2 removed up to 65% pyritic sulphur and up to 6% organic sulphur after 30 days incubation. These cultures HB1 and HB2 differed slightly in their abilities to remove both pyritic and organic sulphur.
Less organic sulphur was removed when "fresh" Benneydale coal was inoculated for 30 days than was the case using pretreated Benneydale coal. In previous experiments with pretreated Benneydale coal, HB1 was able to remove (14-15)% of organic sulphur and HB2 removed (9-11)% (page 40). Pretreatment of the coal probably enhanced subsequent microbial attack; attributable to removal of acid-consuming material or toxic elements as suggested by Silverman *et al.* (1961) and Detz and Barvinchak (1979). They reported that in some cases, acidic prewashing of coal improved the biodesulphurisation rate and also the biological and chemical reactions which happened during the pyritic sulphur removal by *Thiobacillus ferrooxidans* . Acid washing appeared to give more accessibility to microorganisms and their enzymes.

Table 3.7.

Sulphur assay before and after experiments with < 63 μm fresh Benneydale coal at 37° C

No Exp	Culture	Sulphur assay (%) Before test				Sulphur assay (%) After test				Days	% Tot.S removal	% Py.S removal	% Org.S removal	Rate Org.S Removal mg/l.day
		S_{tot}	S_{Sulp}	S_{pyr}	S_{org}	S_{tot}	S_{Sulp}	S_{pyr}	S_{org}					
J1	HB1	2.64	0.03	1.08	1.53	1.64	0.12	0.16	1.35	30	38	85	12	5.3
J2	HB1	2.64	0.03	1.08	1.53	1.69	0.15	0.18	1.36	30	36	83	11	5.0
K1	HB2	2.64	0.03	1.08	1.53	1.99	0.07	0.48	1.44	30	25	56	6	2.6
K2	HB2	2.64	0.03	1.08	1.53	1.95	0.13	0.38	1.44	30	26	65	6	2.6

S_{tot} = total sulphur
S_{pyr} = pyritic sulphur
S_{sulp} = sulphate sulphur
S_{org} = organic sulphur

Benneydale coal, particle size < 63 μm

Conditions: air flow rate: 1.5 litre/minute; temperature: 37° C; stirrer speed: 125 rpm

A study of Stranberg and Lewis (1986) demonstrated that pretreatment with nitric acid significantly enhanced the susceptibility of a variety of coals to microbial solubilisation. This type of pretreatment may increase the *"oxidation state"* of coals (Ignasiak *et al.* 1972), but changes in the chemical coal structure which enhance susceptibility are unknown.

The ability to degrade both forms of sulphur (pyritic and organic sulphur) suggests that there are two different groups of microorganism(s) involved in complete sulphur degradation. One group is responsible for pyritic sulphur removal and the other for organic sulphur degradation. As has been indicated in chapter 2, the consortium of microorganisms developed in media H1 and H2 was a complex microbial community, consisting of several fungi and bacterial species together with unidentified organisms probably bacteria. It is reasonable to suppose that the microorganisms which are associated with pyritic sulphur removal are probably dominated by *Thiobacillus ferrooxidans* . In a medium without any pyritic sulphur they are unable to grow. They do not die although they survive, while awaiting the right conditions for growth. When suitable conditions occur, in this case when pyritic sulphur becomes available again, the microbes grow again and consume the pyritic sulphur as their source of energy. This is shown by the pH changes in the growth medium (see table 3.8.). During experiments, using "fresh" Benneydale coal, the pH of the medium decreased more than that of the medium using pretreated Benneydale coal.
Figure 3.4. illustrates the pH changes in the medium using "fresh" Benneydale coal compared with that using pretreated Benneydale coal, for the culture HB1.
Figure 3.5. illustrates the similar changes for using the culture HB2.

Table 3.8.

pH changes for experiments J1-J2; K1-K2; L1-L4; M1-M4.

Day	*pH of experiment*											
	J1	J2	K1	K2	L1	L2	L3	L4	M1	M2	M3	M4
1	3.05	3.06	3.53	3.11	3.11	3.39	3.09	2.99	3.11	3.04	3.28	3.24
2	3.08	3.15	3.56	3.13	3.17	3.42	3.18	2.98	3.13	3.04	3.28	3.23
3	3.13	3.18	3.59	3.15	3.17	3.44	3.17	2.98	3.17	3.04	3.27	3.21
4	3.09	3.14	3.57	3.17	3.16	3.46	3.04	2.95	3.14	3.05	3.23	3.20
5	3.04	3.07	3.50	3.14	3.11	3.41	3.00	2.91	3.07	3.08	3.22	3.18
6	2.97	3.01	3.43	3.12	3.06	3.38	2.99	2.88	3.05	3.08	3.21	3.16
7	2.90	2.95	3.36	3.07	3.03	3.29	2.98	2.84	3.04	3.07	3.21	3.14
8	2.82	2.89	3.24	3.05	2.99	3.20	2.92	2.81	3.01	3.05	3.20	3.12
9	2.76	2.82	3.20	3.04	2.97	3.12	2.87	2.78	2.99	3.03	3.18	3.10
10	2.69	2.75	3.10	3.00	2.93	3.08	2.79	2.75	2.96	3.01	3.17	3.09
11	2.61	2.71	3.08	2.98	2.90	3.02	2.73	2.73	2.90	3.01	3.15	3.08
12	2.53	2.66	2.86	2.96	2.86	2.99	2.64	2.69	2.88	3.00	3.14	3.07
13	2.45	2.64	2.75	2.95	2.82	2.95	2.60	2.62	2.84	2.98	3.13	3.06
14	2.45	2.60	2.75	2.92	2.80	2.91	2.57	2.61	2.79	2.98	3.12	3.06
15	2.36	2.55	2.68	2.90	2.74	2.83	2.51	2.55	2.76	2.95	3.12	3.04
16	2.29	2.50	2.60	2.88	2.72	2.75	2.48	2.51	2.71	2.94	3.11	3.02
17	2.22	2.48	2.52	2.85	2.66	2.67	2.42	2.45	2.69	2.93	3.10	3.00
18	2.16	2.43	2.46	2.79	2.62	2.60	2.39	2.41	2.66	2.90	3.10	3.00
19	2.12	2.40	2.42	2.76	2.57	2.52	2.35	2.32	2.64	2.89	3.09	2.98
20	2.06	2.39	2.42	2.71	2.54	2.46	2.27	2.28	2.61	2.88	3.09	2.96
21	2.06	2.30	2.40	2.70	2.50	2.44	2.21	2.25	2.60	2.88	3.08	2.94
22	2.05	2.27	2.40	2.66	2.44	2.35	2.16	2.20	2.56	2.85	3.08	2.92
23	2.05	2.15	2.39	2.64	2.38	2.28	2.12	2.18	2.51	2.85	3.06	2.89
24	2.04	2.10	2.39	2.60	2.34	2.23	2.10	2.15	2.47	2.85	3.05	2.87
25	2.04	2.10	2.38	2.60	2.30	2.19	2.08	2.12	2.46	2.84	3.05	2.86
26	2.04	2.08	2.38	2.58	2.28	2.18	2.06	2.08	2.45	2.84	3.04	2.86
27	2.04	2.08	2.38	2.55	2.28	2.18	2.04	2.06	2.45	2.82	3.04	2.85
28	2.03	2.07	2.38	2.55	2.26	2.16	2.04	2.04	2.42	2.82	3.02	2.84
29	2.03	2.07	2.38	2.55	2.25	2.16	2.04	2.01	2.42	2.82	3.02	2.84
30	2.03	2.07	2.38	2.55	2.25	2.16	2.04	2.01	2.42	2.82	3.02	2.84
$\%[H^+]$ gain	947	877	1312	263	624	1598	1022	855	390	68	82	152

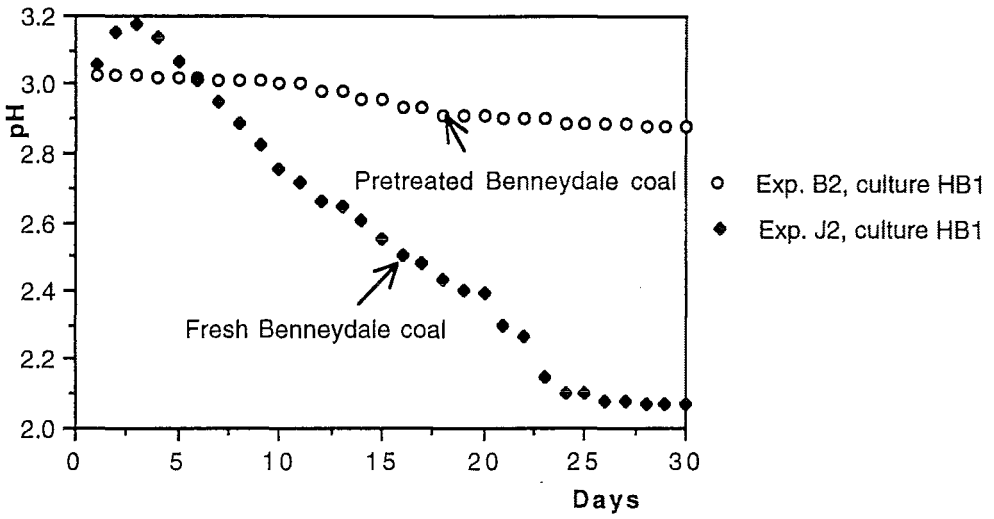


Figure 3. 4.: pH changes over time for culture HB1 with "fresh" and pretreated Benneydale coal.

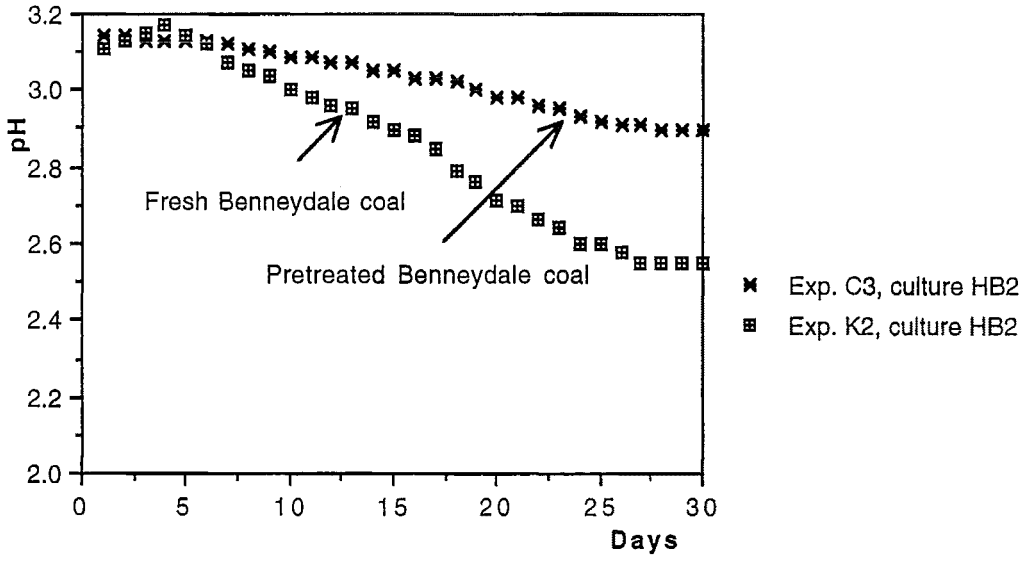


Figure 3. 5.: pH changes over time for culture HB2 with "fresh" and pretreated Benneydale coal.

Both figures show that using "fresh" Benneydale coal the pH of the medium undergoes a significantly decrease than was the case for pretreated Benneydale coal. This indicated that more acid was produced in the experiments with "fresh" Benneydale coal due to the greater availability of pyritic sulphur as the energy source.
There is no doubt that collectively the microbes have a definite capacity to remove both forms of sulphur. This capacity will be discussed further in the next section.

Desulphurisation at different temperatures

Experiments at 32º, 42º, 47º and 52ºC with an air flow rate of 1.5 litre/minute and stirrer speed of 125 rpm.

The result of the experiments at 37ºC using "fresh" Benneydale coal revealed that the cultures HB1 and HB2 could remove both forms of sulphur from coal, although they differed in their efficiency of sulphur removal. The previous result also suggested there were two different groups of microorganisms involved in sulphur removal, one group dealing with pyritic sulphur and another group responsible for organic sulphur removal. This was tested in another set of experiments conducted at different temperatures 32º; 42º; 47º and 52ºC.

The conditions in the reactor were the same as used previously (air flow rate: 1.5 litre/minute; stirrer speed: 125 rpm).
The "fresh" Benneydale coal was ground to < 63 µm.

Table 3. 9. shows the results of these experiments

Table 3.9.

Sulphur assay before and after experiments with < 63 μm fresh Benneydale coal at at different temperature

No Exp	T °C	Culture	Sulphur assay (%) Before test				Sulphur assay (%) After test				Days	% Tot.S removal	% Py.S removal	% Org.S removal	Rate Org.S Removal mg/l.day	Rate Py.S Removal mg/l.day
			S_{tot}	S_{Sulp}	S_{pyr}	S_{org}	S_{tot}	S_{Sulp}	S_{pyr}	S_{org}						
J2	37	HB1	2.64	0.03	1.08	1.53	1.69	0.15	0.18	1.36	30	36	83	11	5.0	26.7
L1	32	HB1	2.64	0.03	1.08	1.53	1.80	0.08	0.32	1.41	30	32	70	8	3.5	22.0
L2	42	HB1	2.64	0.03	1.08	1.53	1.68	0.13	0.20	1.36	30	36	81	11	5.0	26.0
L3	47	HB1	2.64	0.03	1.08	1.53	1.73	0.34	0.27	1.12	30	35	75	27	11.9	23.5
L4	52	HB1	2.64	0.03	1.08	1.53	1.96	0.49	0.30	1.16	30	25	72	24	10.7	22.5
K2	37	HB2	2.64	0.03	1.08	1.53	1.95	0.13	0.38	1.44	30	26	65	6	2.6	20.2
M1	32	HB2	2.64	0.03	1.08	1.53	2.15	0.08	0.59	1.48	30	19	45	3	1.4	14.1
M2	42	HB2	2.64	0.03	1.08	1.53	2.16	0.08	0.65	1.43	30	18	40	6	3.0	12.9
M3	47	HB2	2.64	0.03	1.08	1.53	2.19	0.14	0.66	1.39	30	17	39	9	4.1	12.4
M4	52	HB2	2.64	0.03	1.08	1.53	2.13	0.13	0.61	1.39	30	22	43	9	4.0	13.6

S_{tot} = total sulphur
S_{pyr} = pyritic sulphur
S_{sulp} = sulphate sulphur
S_{org} = organic sulphur

(a) Benneydale coal, particle size < 63 μm.

Conditions: air flow rate: 1.5 litre/minute; temperature: (32-52)°C; stirrer speed: 125 rpm

Pyritic sulphur removal in relation to temperature

Essentially the ability of each culture (HB1 and HB2) to remove pyritic sulphur is affected by the temperature.
In table 3.9. the culture HB1 could remove (70-83)% of the pyritic sulphur, but HB2 removed only (39-65)%. Both cultures, HB1 and HB2, performed best at 37°C temperature, where HB1 removed 83% of pyritic sulphur and HB2 removed 65% after 30 days of incubation.
At 32°C, as shown by experiments L1 and M1 (table 3.9), HB1 removed only 70% of pyritic sulphur whilst HB2 removed 45% .
At 42°C (see experiments L2 and M2, table 3.9): HB1 degraded 81% but HB2 degraded only 40% pyritic sulphur.
Further elevation of temperature to 47°C led to a further decrease in pyritic sulphur removal by both HB1 and HB2. Only 75% of pyritic sulphur was removed by HB1. The performance of HB2 was even worse, removing 39% pyritic sulphur (see experiments L3 and M3, table 3.9).
At 52°C (experiments L4 and M4, table 3.9) HB1 removed 72% of pyritic sulphur compared with 43% by HB2.

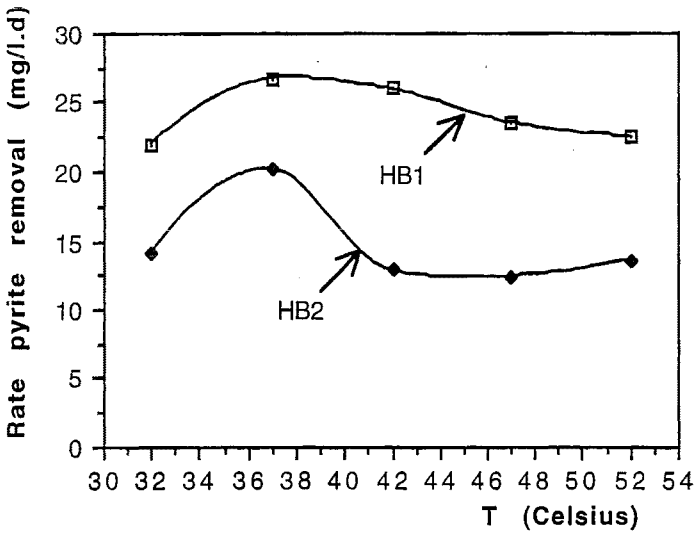


Figure 3. 6.: Effect of temperature on rate of pyritic sulphur removal

Figure 3. 6. shows the profile of the rate of pyritic sulphur removal by HB1 and HB2 in relation to temperature. It shows the maximum rate of pyritic sulphur removal was achieved at 37°C and further increases of temperature led to a decrease in pyritic sulphur removal. This profile

indicates that the most suitable temperature for both of the cultures HB1 and HB2 to remove pyritic sulphur was 37°C.

Organic sulphur removal in relation with temperature

The results of these experiments are shown in figure 3.7.

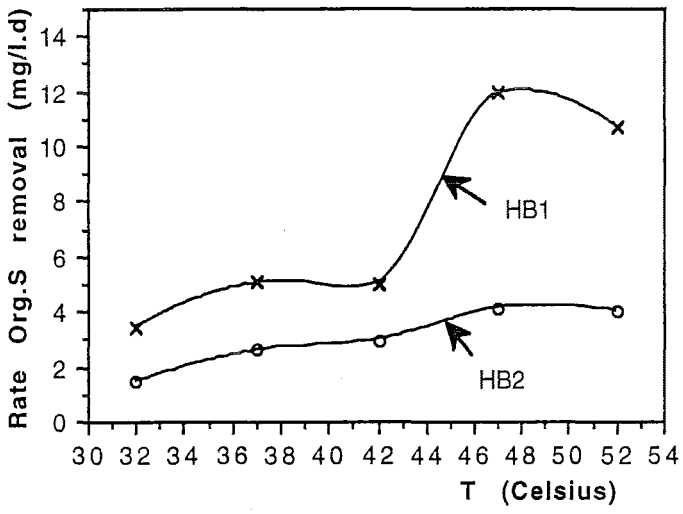


Figure 3. 7. Effect of temperature on rate of organic sulphur removal

This figure indicates that temperature greatly affects the capability of the cultures HB1 and HB2 in degrading organic sulphur and 47°C is the optimum temperature for both cultures.

The patterns of organic sulphur removal in relation to temperature are different from those observed for pyritic sulphur. Both cultures HB1 and HB2 indicate that their ability to remove organic sulphur improves with increasing temperature.

At 32°C, HB1 removed 8% of organic sulphur and HB2 removed 3% of organic sulphur (see experiments L1 and M1, table 3.9). This was much lower than sulphur removal occurring at 37°C (see experiments J2 and K2, table 3.9) which showed up to 11% and 6% organic sulphur reduction for the cultures HB1 and HB2 respectively.

At 42°C, the performance of both cultures (experiments L2 and M2, table 3.9) increased slightly. HB1 removed up to 11% organic sulphur and HB2 removed up to 7% of organic sulphur. When temperature was increased to 47°C, their performance in terms of organic sulphur removal increased quite significantly, particularly for the culture HB1 (see

experiments L3 and M3, table 3.9). The data showed that this culture removed up to 27% of organic sulphur and that culture HB2 removed up to 9% of organic sulphur. Experiments at the highest temperature of 52°C (see experiments L4 and M4, table 3.9) indicated decreasing efficiency with HB1 removing 24% of organic sulphur compared with 9% removal by HB2.

Microorganisms involved

Both figures (figure 3.6. and figure 3.7.) illustrate that the optimum rate of pyritic sulphur and organic sulphur removal for both of the cultures HB1 and HB2 was achieved at different temperatures.
The optimum temperature for pyritic sulphur removal was 37°C and for organic sulphur removal it was 47°C. This result supports the previous finding that there were two entirely different groups of microorganisms, one group responsible for pyritic sulphur degradation and another group responsible for organic sulphur removal (page 50). The first group attacked the pyritic sulphur best at 37°C, whilst the other probably altered the coal structure in order to access and utilize the organically bonded sulphur in the coal matrix.
As shown earlier, 35° to 40°C was also the most suitable working temperature range for *Thiobacillus ferrooxidans* (see part I-chapter 3.2.3). It is possible, that the mixed culture responsible for pyritic sulphur removal was dominated by the adapted *Thiobacillus ferrooxidans* descendant which was used in pyritic sulphur removal. *Thiobacillus ferrooxidans* (and its descendant) is a robust bacterium and it is reasonable to suppose that it can survive even in unfavourable medium i.e. sulphur deficient. When its environment again becomes favourable due to the avialability of pyritic sulphur, it becomes active again and starts to degrade pyritic sulphur. However its' performance decreases over time due to unknown factors.

The data in table 3. 10. show that the rate of pyritic sulphur removal by *Thiobacillus ferrooxidans* is clearly higher (62.6 $mg.l^{-1}.day^{-1}$) than the rate of pyritic sulphur removal by the culture HB1 (26.7 $mg.l^{-1}.day^{-1}$) or the culture HB2 (20.2 $mg.l^{-1}.day^{-1}$).

Table 3.10

Rate of pyritic sulphur removal from coal by microbes

Culture	Rate of pyritic S removal	Conditions
Thiobacillus ferrooxidans dominant	62.6 $mg.l^{-1}.day^{-1}$	15% w/v coal 40°C air flow: 1.5 litre/minute stirrer speed: 125 rpm
HB1	26.7 $mg.l^{-1}.day^{-1}$	10% w/v coal 37°C air flow: 1.5 litre/minute stirrer speed: 125 rpm
HB2	20.2 $mg.l^{-1}.day^{-1}$	10% w/v coal 37°C air flow: 1.5 litre/minute stirrer speed: 125 rpm

Gokcay and Yurteri (1983) also claimed that a mesophilic *Thiobacillus ferrooxidans* strain TH1 removed both forms of sulphur from Turkish lignite at 50°C and pH 3.0 (see chapter 1-literature review). However, it is too early to conclude that the same organism is responsible for this sulphur removal from Benneydale coal. Further work is needed employing a pure culture isolated from the effluent of the experiments depicted in table 3. 10.

Since *Thiobacillus ferrooxidans* does not remove organic sulphur from coal, it is likely that there is an entirely different type of organism responsible for organic sulphur removal. This organism has optimum temperature of 47°C and its performance at 52°C is slightly impaired. In contrast, when temperature exceeds 37°C, the organism(s) responsible for pyritic sulphur degradation become impaired as shown by the decrease in the percentage of pyritic sulphur removal. This difference in working

temperature suggests that the microorganisms responsible for organic sulphur degradation are not the same as those responsible for pyritic sulphur degradation. At present, the microorganisms responsible for organic sulphur removal have not yet been isolated and characterized.

3.4.3. CONCLUSION

There is good reason to suppose that there were several organisms working together (symbiosis) to degrade both types of sulphur from coal. They, collectively, had the capability of degrading both types of sulphur and probably broke down the coal structure. Some organisms used organic sulphur, while others degraded pyritic sulphur. Degradation of sulphur might occur through several routes. Based on recent work involving microbial communities, it seemed likely that several organisms were directly involved in sulphur mobilization, while other organisms were needed to fulfill nutrient requirements (Ward *et al.* 1987). Similar systems probably occurred in this study, although the steps and substances released were not the same. At present, this speculation is not confirmed and further work in this direction is required.

First of all, more microbiological experiments are needed to isolate and characterize pure cultures from the effluent. Pure cultures of each organism could be tested for their ability to degrade a range of organic sulphur compounds.

The next step would be to ascertain the optimum conditions for microbial growth and sulphur removal. This could be done in conjunction with improvements in reactor design.

Future work would therefore address a variety of problems including complete sulphur removal and solubilization of coal as a means of transforming solid coal to more usable liquid forms with low sulphur content.

3.5. EXPERIMENTS WITH NEW CREEK COAL

3.5.1. EXPERIMENTAL

In this section, the capability of HB1 and HB2 to degrade organic sulphur on another type of coal was determined. The coal, New Creek coal from the Buller area, West Coast, South-Island, New Zealand, which contains high organic sulphur (5%) was ground to particle size < 105 μm. To determine the effect of coal particle size on the rate of desulphurisation additional experiments were performed using coal of particle size < 152μm. These experiments were conducted at 37°C. In addition effect of temperature (32° to 52°C) on the rate of organic sulphur removal was also examined.

Coal:

New Creek coal contains relatively high organic sulphur (5.0%) compared with the Benneydale coal (1.2%). Table 3.11. shows the proximate analysis of New Creek and Benneydale coal.

Table 3.11

Proximate analysis for New Creek and Benneydale coal.

Composition	New Creek coal	Benneydale coal
Ash	2.6 %	6.5 %
Moisture content	17.8 %	22.2 %
Volatile matter	37.4 %	35.6 %
Fixed carbon	42.3 %	35.7 %
Sulphur content	5.13 %	2.10 %
Gross calorific value	10,400 BTU/lb	9,270 BTU/lb

Experimental conditions:

The following conditions were used:

air flow rate: 1.5 litre/minute,

temperature: 32°, 37°, 42°, 47°, and 52°C,

stirrer speed: 125 rpm.

The initial pH of the medium was adjusted to the desired pH with 2.0 M HCl or 2.0 M NaOH.

Incubation lasted 25 days.

3.5.2. RESULTS AND DISCUSSION

The experiments demonstrated that the mixed acidophilic cultures, HB1 and HB2, were capable of degrading organic sulphur from New Creek coal.

Table 3.12. summarizes the result of the experiments at different temperatures within the range of 32°C to 52°C. Two other experiments (N6 and O6) determined the performance of the same cultures (HB1 and HB2) using a larger particle size of coal substrate.

Figure 3.8. shows the pattern of the rate of organic sulphur removal from New Creek coal as a function of temperature. It indicated that the optimum rate of organic sulphur removal was achieved at 47°C. This result supports the previous work that the optimum working temperature for HB1 and HB2 for organic sulphur degradation was 47°C (see chapter 3.4., more specifically figure 3.7.).

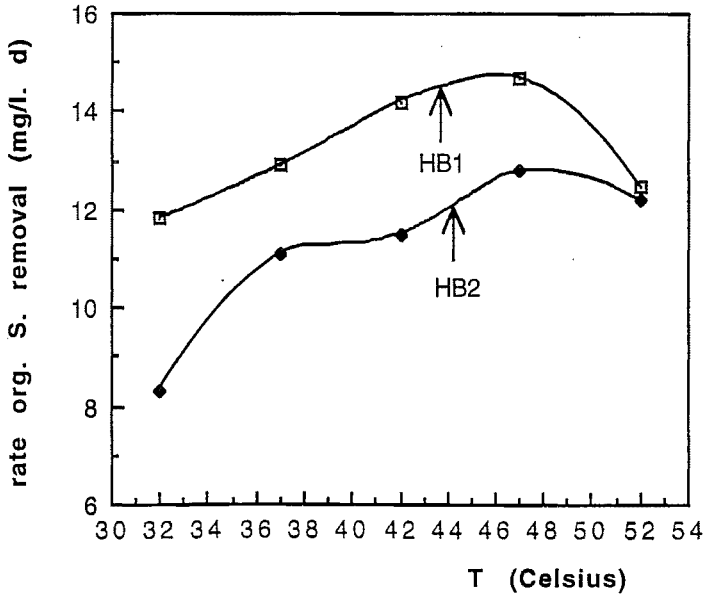


Figure 3. 8. Effect of temperature on rate of organic sulphur removal for New Creek coal.

Table 3.12.

Sulphur assay before and after experiments using New Creek coal.

No Exp.	Culture	T °C	Sulphur assay (%) Before test				Sulphur assay (%) After test				Incubation (days)	% Tot S removal	% Org S removal	Rate Org.S Removal mg/l.day
			S_{tot}	S_{Sulp}	S_{pyr}	S_{org}	S_{tot}	S_{Sulp}	S_{pyr}	S_{org}				
N1a	HB1	32	5.13	0.01	0.07	5.04	4.89	0.04	0.13	4.72	25	5	6	11.8
N2a	HB1	37	5.13	0.01	0.07	5.04	4.90	0.06	0.17	4.67	25	4	7	12.9
N3a	HB1	42	5.13	0.01	0.07	5.04	4.89	0.08	0.18	4.63	25	5	8	14.2
N4a	HB1	47	5.13	0.01	0.07	5.04	4.82	0.04	0.16	4.62	25	6	8	14.7
N5a	HB1	52	5.13	0.01	0.07	5.04	4.88	0.02	0.18	4.68	25	5	7	12.5
N6b	HB1	37	5.13	0.01	0.07	5.04	5.04	0.04	0.19	4.81	25	2	5	6.9
O1a	HB2	32	5.13	0.01	0.07	5.04	5.02	0.04	0.17	4.80	25	2	5	8.3
O2a	HB2	37	5.13	0.01	0.07	5.04	4.95	0.05	0.17	4.72	25	3	6	11.1
O3a	HB2	42	5.13	0.01	0.07	5.04	4.96	0.09	0.16	4.71	25	3	6	11.5
O4a	HB2	47	5.13	0.01	0.07	5.04	4.90	0.00	0.23	4.67	25	4	7	12.8
O5a	HB2	52	5.13	0.01	0.07	5.04	4.83	0.03	0.12	4.69	25	6	7	12.2
O6b	HB2	37	5.13	0.01	0.07	5.04	5.03	0.02	0.10	4.90	25	2	3	4.1

S_{tot} = total sulphur
S_{pyr} = pyritic sulphur
S_{sulp} = sulphate sulphur
S_{org} = organic sulphur

(a) New Creek coal, particle size < 105 µm

(b) New Creek coal, particle size < 152 µm

Conditions: air flow rate: 1.5 litre/minute; temperature: 37° C to 52° C; stirrer speed: 125 rpm

To check the effect of particle size on the rate of organic sulphur removal further experiments, N6 and O6, were performed. The particle size of coal substrate in these experiments was < 152 μm. The rates of organic sulphur removed were 6.9 $mg.l^{-1}.d^{-1}$ and 4.1 $mg.l^{-1}.d^{-1}$, for HB1 and HB2 respectively. For comparison the rate of organic sulphur removal from the experiment with smaller coal particle size, < 105 μm was 12.9 $mg.l^{-1}.d^{-1}$ for HB1 and 11.1 $mg.l^{-1}.d^{-1}$ for HB2 (see experiments N2 and O2, table 3.12).
The reduction of the particle size of coal substrate from 152 μm to 105 μm increased the rate of organic sulphur removal by factors of 1.9 and 2.7, for cultures HB1 and HB2, respectively. This result supports the earlier findings that the rate of organic sulphur removal using HB1 and HB2 correlates with the particle size (page 43).

Table 3.13. shows the rate of organic sulphur removal from New Creek coal compared with the rate observed from Benneydale coal (the reactor conditions were the same in both cases: air flow rate of 1.5 litre/minute; stirrer speed of 125 rpm and temperature of 37°C).

Table 3.13.

Organic sulphur removal for pretreated Benneydale coal and New Creek coal

Type of coal	Rate org.S removal ($mg.l^{-1}.day^{-1}$)		% organic sulphur removal	
	HB1	HB2	HB1	HB2
New Creek				
particle size <105μm	12.9	11.1	7.3	6.3
particle size <152μm	6.9.	4.1	4.6	2.8
Pretreated Benneydale(*)				
particle size <105μm	3.4	3.3	8.7	7.3
particle size <152μm	2.7	1.7	7.0	4.0

(*) See table 3.3.

The data in table 3.13. indicated that the rate of organic sulphur removal in the experiments with New Creek coal was higher than the rate found for pretreated Benneydale coal, by a factor within the range of (2.4-2.5) and (3.4-3.8), for particle size < 152 μm and < 105 μm, respectively.
The organic sulphur content in New Creek coal is three times higher than that of Benneydale coal, so it is to be expected that the rate of organic sulphur removal in experiments using New Creek coal should be higher than the rates obtained with pretreated Benneydale coal. The result in table 3.13. supports this view, however, in terms of the amount of organic sulphur removal (percentage), the experiment with New Creek coal was less effective than that with the Benneydale coal. Boateng and Phillips (1976) in their examination of coal structure using optical and scanning electron microscopy and electron microprobe showed that organic sulphur was evenly distributed in coal. In this case, the variation in organic sulphur degradation seems to be more a function of the type of coal than of the specific microorganisms. This is also indicated by other researchers, such as, Scott *et al.* (1986); Klein *et al.* (1988) and Khalid *et al.* (1990). Observation of the composition of the coal (see table 3.11.) shows that New Creek coal is different from Benneydale coal. One important indication is the moisture content of the coal. The moisture content in New Creek coal is 17.8% compared with 22.2% in Benneydale coal, which indicates that New Creek coal is more compact than Benneydale coal. The moisture content of coal represents the degree of compactness which has occurred during the formation of the coal. High temperature and pressure are the main factors affecting the degree of compactness. Compactness relates closely to the distribution of pores and the pore size within the coal itself. If the coal is more compact, the coal porosity decreases and consequently the accessibility for gases, liquids or microbial attack will be reduced (see table 3.6). The fixed carbon content (42.3% for New Creek coal compared with 35.7% for Benneydale coal) and the gross calorific value (10,400 BTU/lb for New Creek coal and 9,270 BTU/lb for Benneydale coal) also indicate that New Creek coal belongs to a higher rank of coal than Benneydale coal. Usually a coal from a higher rank is more mature and compact than one from a lower rank. Consequently, New Creek coal is less susceptible to microbial attack and more difficult to break down as shown in the present study.

3.5.3. CONCLUSION

Both the mixed cultures, HB1 and HB2, showed that they were capable of degrading organic sulphur from different types of coal. They were able to degrade organic sulphur from a higher rank coal, in this case from New Creek coal, although the rate of organic sulphur removal was slower than in lower rank coal. The more compact the coal, the less accessible it is to microbial attack, making degradation correspondingly more difficult. Accessibility to the interior of the coal can be improved by reducing the particle size. It can be concluded that microbial organic sulphur removal using these acidophilic cultures, HB1 and HB2, should be tailored to each type of coal substrate.

3.6. MEDIA COMPOSITION AND THEIR ROLE IN SULPHUR REMOVAL

Nutrients are an important aspect affecting the growth of microorganism. The role of nutrients in this investigation has been little studied. However, many authors such as Hoffman *et al.* (1981) and Kargi and Robinson (1985), agreed that complex organic nutrients and mineral salts significantly affected the rate and extent of coal desulphurisation using *Thiobacillus ferrooxidans.*

A review of the literature was undertaken in order to design media which would allow the optimal growth and subsequent organic sulphur removal by the mixed cultures HB1 and HB2.

3.6.1. MEDIA COMPOSITION

In this study two kinds of medium (H1 and H2) were used to study the degradation ability of the isolated acidophilic cultures, HB1 and HB2.
Table 3.14. shows the composition of media H1 and H2.

Table 3.14

Composition of media and ratios N/P and N/Mg

Composition of medium H1	Composition of medium H2*
NH_4Cl: 0.8 g/l	$MgCl_2.6H_2O$: 0.2 g/l
KH_2PO_4: 0.4 g/l	KNO_3: 1.0 g/l
$MgNO_3.6H_2O$: 0.2 g/l	$CaCl_2.2H_2O$: 0.1 g/l
	$FeCl_3.6H_2O$: 0.01 g/l
	K_2HPO_4: 2.0 g/l
Ratio:	Ratio:
N/P= 2.5	N/P= 0.4
N/Mg= 10.2	N/Mg= 5.8

(*) Medium H2 was slightly modified from the original medium described by Van Afferden (1990).

Table 3.14. shows that the media are different, either in the type or amounts of salts. Some literature dealing with pyritic sulphur removal (see below) indicated that ammonium and phosphate content were the

most important factors, that should be considered in any desulphurisation of coal. For example, in a study on the effect of nutrient concentrations on the microbiological leaching of zinc sulphide, Torma *et al.* (1970) demonstrated that the ammonium concentration controlled yield and phosphate concentration affected the rate of desulphurisation. In 1981, Hoffman *et al.* reported that the most effective medium for *Thiobacillus ferrooxidans* in pyritic sulphur oxidation contained relatively low amounts of phosphate (0.7 mM), with an optimal nitrogen/phosphorous ratio (90:1). A study of Kargi and Robinson (1985) also indicated that the microbial desulphurisation rate using thermophilic *Sulfolobus acidocaldarius* was improved nearly ten fold by adjusting the N/P and N/Mg ratios in the nutrient medium. In their study, the maximum rate of pyritic sulphur removal was at N/P = 47.5 and N/Mg = 32 (see table 3.15.). Moreover, all the authors agreed that by keeping the N/P and N/Mg ratios at their optimal level, further improvement in the rate of pyritic sulphur removal was achieved.

Table 3.15.(*)

The influence of N/P and N/Mg ratios on removal of pyritic sulphur from coal

N/P	N/Mg	Rate (mgS/Lh)	% Pyritic S removal
54.8	22.0	17.4	88.1
5.5	22.0		31.7
30.1	36.4	15.6	92.1
30.1	7.3	22.1	84.1
47.5	32.0	33.0	74.6
47.5	11.5	27.5	88.1
12.7	32.5	9.9	24.6
12.7	11.5	14.3	54.8
30.1	21.9	18.2	61.9

(*) Kargi and Robinson, 1985.

With regard to the composition of the media H1 and H2, the data in table 3.14 shows that the N/P ratios were 2.5 and 0.4, and the N/Mg ratios were 10.2 and 5.8 , for medium H1 and H2, respectively. The ratio of N/P and N/Mg in medium H1 and H2 are lower than the ratio N/P and N/Mg

shown in table 3.15. This may be one of the reasons for the limited rate of organic sulphur removal in this study.

Sulphate is abundant in the medium used in the experiment examining pyritic sulphur removal (see part I). In contrast, there is no sulphate in media H1 and H2, so in this case, it is quite reasonable to expect that the rate of pyritic sulphur removal by the culture HB1 and HB2 in this study would be lower than the rate of pyritic sulphur removal in the experiment using *Thiobacillus* mixed culture as shown in part I. The result of the experiments using "fresh" Benneydale coal in chapter 3.4. supports this expectation. However, further studies are needed to investigate the role of sulphate in organic sulphur removal.

In media H1 and H2 chloride is abundant, although the exact concentration of chloride to promote better growth and hence higher organic sulphur removal is still unknown. A study by Tuovinen *et al.* (1971) showed that if chloride concentration was high there was a significant decrease in the rate of iron oxidation, although microorganisms would be able to tolerate higher chloride concentration after step-wise adaptation periods, as reported by Mayling in 1966.
It is suggested that reduction in chloride concentration in medium H1 and H2 could lead to an improvement in the ability of the isolated cultures HB1 and HB2 in degrading sulphur.

Potassium and calcium are probably required in trace amounts. The need for potassium as well as for calcium is so slight that those cations are supplied in normal circumstances by residues in reagents and glass-ware, and especially in the natural environment by water and soil minerals as well as coal.

It appears that, given the correct nutrient medium, the capacity of the cultures, HB1 and HB2, to degrade organic sulphur may increase, although the forms in which the important elements may be utilized could vary greatly from one type of microorganism to another.

3.6.2. CONCLUSION

In this study the effect of certain nutrients on organic sulphur removal was extensively studied. Basically the composition of media H1 and H2 is different. Medium H2 has an N/P ratio = 0.4 and N/Mg ratio = 5.8 compared with N/P ratio = 2.5 and N/Mg ratio = 10.2 in medium H1. The lower N/P and N/Mg ratios in medium H2 is probably one of the reasons for the lower rate of organic sulphur removal by the culture HB2. It is possible that an increase in N/P and N/Mg ratios in media H1 and H2 would improve their efficiency i.e. contribute to increase levels of organic sulphur removal.

Up to the present study, the importance of nutrient composition in microbiological organic sulphur removal has been very little examined, and further investigations are needed to gain better understanding of the role of nutrients in desulphurisation processes.

CHAPTER 4

RECOMMENDATIONS FOR FURTHER WORK

The mixed acidophilic cultures, HB1 and HB2, enriched by a simple selective culture method, have demonstrated their capability of degrading organic sulphur in coal. Although, these microorganisms are not yet completely characterized, the result are encouraging.

These cultures, HB1 and HB2, are consortia of microorganisms. The identification of these consortia, revealed that they consisted at least of cocci bacteria and bacteria of the *Bacillus* species, with some *Aspergillus* and *Penicillium* fungal species. There is good reason to suppose that these organisms collectively have the ability to degrade both forms of sulphur from coal and of breaking down the coal. Understanding the interactions of microbial populations in this acidophilic environment is important for evaluating factors influencing the desulphurisation. So far the role of individual organisms is still uncertain. Further study should be directed towards ascertaining which particular organism(s) has the capability of removing organic sulphur and of isolating these microbes in pure culture for further intensive study.

A preliminary investigation of the role of fungi in solubilizing coal indicated that the fungi found in this study might have this capability. In tests, the *Penicillium* sp. indicated that it could solubilize the coal substrate to a liquid form when cultured on the surface of pretreated Benneydale coal. This phenomenon is of sufficient interest to encourage a more serious study on the role of fungi, in particular the *Penicillium* sp., for biosolubilisation of coal, with the objective of converting solid

coal into a more readily usable liquid form and at the same time reducing its sulphur content.

Although it has been demonstrated that organic sulphur from different types of coal can be removed by microbial action (cultures, HB1 and HB2) the process is not clearly understood and the end products have not been clearly identified. The results obtained to date are intriguing, but additional research will be needed to completely define the system.

Improvements in organic sulphur analytical methods are needed, especially for the bioprocess monitoring of organic sulphur removal. The measurement of organic sulphur by difference (total sulphur minus pyritic sulphur and sulphate sulphur), potentially leads to some discrepancies, making the interpretation of the biological removal of organic sulphur difficult. Recent advances in coal characterization by Fourier transformation infra-red spectroscopy, scanning microscopy coupled with energy dispersive X-ray microanalysis and others may hold promise.

The study also shows that the rate of desulphurisation increased with reduction of the coal particle size. Technically, coal can be ground to 2 μm which is an ideal size for screening microorganisms (Fakoussa and Truper, 1983), but this grinding process is relatively expensive considering the value of coal itself. Coal is relatively abundant and cheap and there is still an enormous amount of coal with low sulphur content; as long as economic considerations are the main factor, the application of organic sulphur removal from coal is still a long way off. The application of this microbial technique needs further study and perhaps it will be more applicable for cleaning petroleum products. Petroleum products are in liquid form which is more acceptable for enzyme reactions.

The degradation of organic sulphur from coal requires the cleavage of covalent bonds in the coal matrix, specifically of aliphatic and ether structures (Klein *et al* . 1988). In order to obtain more information about the mechanism(s) and the pathway(s) of organic sulphur degradation, further experiments in this direction are needed. It may be important, also to determine not only the organic sulphur content of coal but also how organic sulphur occurs.

Because of the complex structure of coal, organic sulphur removal from coal may be best approached by a combination of biological and chemical steps. The chemical steps are directed towards modifying the sulphur bond and the pore structure in the coal matrix to improve its biological availability. However, to find a microorganism, or a consortium/series of microorganisms, which will break down particular bonds or break ring structures, is not likely to be an easy task.

Recent interest and developments in genetic engineering have raised more possibilities; however, the difficulties of growing these acidophilic organisms on agar due to their low-growth pH make many conventional genetic manipulation techniques, such as replica plating and the use of acid-labile plasmid curing agents, difficult. Coal bioprocessing is a new, exciting area that demands the integration of various disciplines. This has led to an increase in cooperation between microbiologists, genetic and chemical engineers in developing bioprocessing systems that are more efficient and cost effective. More interdisciplinary cooperation between microbiologists, chemists, metallurgists, and engineers is necessary to identify, characterize, select and develop bioprocesses for industrial application in the future.

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APPENDIX A

CHARACTERISTIC OF ORGANIC SULPHUR

Sulphur is the second element of the sixth main group of the periodic system. Due to the availability of the d orbital for bounds (as an element of the third period), it can take a number of valences at a number of oxidation states, ranging from -2 to +6. Similarly organic sulphur compounds can also take a number of oxidation states, and in principle a multitude of different reactions with organic sulphur in coal are possible.

ad. 1. Mercaptan or thiol (R-SH)

Organic compounds containing the -SH (sulphydryl) group are known in modern nomenclature as thiols, although the traditional name "mercaptan" is widely and perhaps more commonly used. An important characteristic feature of thiols (R-SH, oxidation states -2) is their reactivity with oxidents. Thiols are readily oxidised to disulphides (oxidation states -1) with mild oxidizing agents.

$$4\ R\text{-}SH + 4\ O_2 \text{ ------> } 2\ R\text{-}SS\text{-}R + 2\ H_2O$$

or via the unstable intermediate sulphenates (R-SOH, oxidation states 0), sulphinates ($R\text{-}SO_2H$, oxidation states +2) and sulphonates ($R\text{-}SO_3H$, oxidation states +4).
Thiols can occur as :
a. alkyl mercaptans (alkane thiols), $R\text{-}CH_2\text{-}CH_2SH$
b. thioglycollic acid (mercaptoacetic acid), $HS\text{-}CH_2\text{-}COOH$
c. thioacetic acid (thiolacetic acid), $C_2H_5\text{-}SH$
d. sulphur-containing amino acid. There are three naturally occurring amino acids : cystine; cysteine; and methionine. Perhaps the thiol of most interest to a biochemist is the amino acid cysteine.
e. aryl mercaptans, such as thiophenol; thiocresols; toluene -3, 4-dithiol; benzyl mercaptan and thionapthols.

ad. 2. Sulphide (thio-ether)

These compounds have the general formula R-S-R. The sulphur of organic sulphides or thio-ethers, respectively, can expand its octet into the d-orbitals. As a result, thio-ethers can be subsequently oxidized to the corresponding sulphoxides and sulphones,

or in other words the oxidation yields sulphoxides which may be further oxidized to sulphones.
Some of sulphides can occur as:
a. dialkyl sulphides (small quantities occur naturally in certain marine algae, in various green plants, and in crude petroleum).
b. benzylphenyl sulphide
c. methylphenyl sulphide
d. phenylalkyl sulphide
e. dibenzyl sulphide.
f. diphenyl sulphide, etc.

ad. 3. Disulphide

These compounds have the general formula R-S-S-R. Organic disulphides are unreactive in comparison to thiols. They can be transferred by a homolytic or heterolytic cleavage into thiols. With regard to organic sulphur removal, disulphides have similar reactions to thiols.
Some examples of this disulphide group are:
diphenyl disulphide, dimethyl disulphide, diethyl disulphide, di-iso-propyl disulphide, etc.

ad. 4. Aromatic systems containing the thiophene rings (heterocyclic sulphur compounds)

Heterocyclic compounds are cyclic (ring) compounds in which the ring members consist of carbon and other elements, the commonest being oxygen, nitrogen, and sulphur. Heterocyclic compounds have five- or six-membered rings, are aromatic in character, and contain conjugated double bonds.
The most important heterocyclic sulphur compounds consist of five-membered rings:
a. with sulphur as the "hetero-atom", the other four being carbon (e.g. thiophene); or
b. with sulphur and nitrogen as "hetero-atoms", the other three being carbon (e.g. thiozole); or
c. either a or b plus a fused benzene ring (benzothiazole).
Perhaps, the most interesting related to coal desulphurisation is the "thiophene" which is found mostly in coal and other related carbon sources such as shale, peat etc.
The thiophenes (cyclic thio-ethers) are often considered as coal-relevant model compounds. Like thio-ethers they can be oxidized biochemically to sulphoxides and sulphones.

APPENDIX B

TYPICAL PARTICLE SIZE DISTRIBUTION RANGE OF COAL (*)

Particle size range (μm)	Coal size < 152 μm (% weight)	Coal size < 105 μm (% weight)	Coal size < 63 μm (% weight)
188.0 - 87.2	19.25	9.05	0.00
87.2 - 53.5	13.50	12.00	9.00
53.5 - 37.6	8.05	5.40	20.70
37.6 - 28.1	6.00	5.05	8.65
28.1 - 21.5	5.05	4.40	4.20
21.5 - 16.7	4.80	3.40	4.95
16.7 - 13.0	5.05	4.25	4.70
13.0 - 10.1	6.00	7.20	4.40
10.1 - 7.9	6.25	7.45	4.50
7.9 - 6.2	5.10	4.75	3.10
6.2 - 4.8	4.50	3.10	2.10
4.8 - 3.8	5.35	5.40	4.25
3.8 - 3.0	10.75	28.40	28.95
3.0 - 2.4	0.25	0.00	0.00
2.4 - 1.9	0.00	0.00	0.00

(*) Particle size analysis was done by using Malvern 2600 D Particle sizer VA. 6, made by Malvern Instruments Ltd, Spring Lane, Malvern, England.

APPENDIX C

COAL WEIGHT, MEDIUM pH AND ORGANIC SULPHUR REMOVAL

Exp.	Coal weight (gr) before	after	Δ weight (gr)	pH medium before	after	Rate Org.S removal (mg/l.day)	% Org.S removal
B1	100.8	92.3	8.5	2.50	2.39	6.14	14.09
B2	101.2	92.9	8.3	3.03	2.88	5.28	13.85
B3	100.5	92.0	8.5	2.43	2.34	5.53	14.62
B4	107.2	96.8	10.4	3.51	3.19	5.59	13.85
B5	90.7	81.3	9.4	3.30	3.08	4.73	13.85
B6	99.0	97.5	1.5	3.03	3.02	0.86	2.16
C1	82.2	74.6	7.6	3.67	3.36	3.35	9.21
C2	99.9	91.5	8.4	3.58	3.29	4.92	11.11
C3	99.9	91.2	8.7	3.14	2.90	4.05	11.11
C4	83.3	75.3	8.0	2.50	2.37	3.86	10.53
C5	92.1	83.7	8.4	3.60	3.24	4.00	10.00
C6	85.5	84.2	1.3	2.50	2.48	0.50	1.44
D1	100.4	92.9	7.5	3.56	3.28	3.49	8.75
D2	95.2	88.8	6.4	2.37	2.21	3.04	8.59
D3	100.5	93.3	7.2	3.07	2.83	3.50	8.53
E1	108.4	102.2	6.2	4.28	3.92	4.40	7.20
E2	90.2	84.5	5.7	3.64	3.31	2.61	7.81
E3	92.0	86.6	5.4	2.43	2.34	2.93	7.38
F1	113.6	110.7	2.9	2.68	2.48	3.29	6.67
F2	78.2	76.8	1.4	3.38	3.10	2.49	7.03
F3	91.5	85.7	5.8	3.13	2.96	2.39	6.98
G1	100.4	96.4	4.0	3.57	3.17	2.33	5.44
G2	99.9	9870	2.9	2.64	2.49	1.45	3.42
G3	81.8	77.1	4.7	2.37	2.21	1.42	4.11
J1	102.2	93.6	8.6	3.05	2.03	5.33	11.76
J2	102.4	94.2	8.2	3.06	2.07	5.05	11.11
K1	100.3	96.3	4.0	3.53	2.38	2.62	5.88
K2	99.6	95.5	4.1	3.11	2.55	2.60	5.88
L1	99.8	93.3	6.5	3.11	2.25	3.47	7.84
L2	101.9	93.1	8.8	3.39	2.16	5.02	11.11
L3	100.3	85.7	14.6	3.09	2.04	11.92	26.80
L4	99.7	90.3	9.4	2.99	2.01	10.69	24.18
M1	99.4	97.0	2.4	3.11	2.42	1.44	3.27
M2	103.2	96.6	6.6	3.04	2.82	2.99	6.54
M3	102.0	94.7	7.3	3.28	3.02	4.14	9.15
M4	99.9	92.2	7.7	3.24	2.84	4.05	9.15

APPENDIX D

pH CHANGES FOR EXPERIMENTS D1-D3; E1-E3; F1-F3, G1-G3.

	pH of experiments											
Day	D1	D2	D3	E1	E2	E3	F1	F2	F3	G1	G2	G3
1	3.56	2.37	3.07	4.28	3.64	2.43	2.68	3.38	3.13	3.57	2.64	2.37
2	3.54	2.36	3.06	4.26	3.63	2.42	2.68	3.36	3.11	3.56	2.64	2.37
3	3.52	2.36	3.04	4.26	3.59	2.42	2.66	3.33	3.09	3.54	2.62	2.36
4	3.50	2.35	3.03	4.24	3.56	2.40	2.66	3.30	3.08	3.53	2.60	2.35
5	3.46	2.35	2.98	4.24	3.53	2.40	2.64	3.29	3.05	3.51	2.58	2.33
6	3.46	2.31	2.98	4.21	3.50	2.39	2.62	3.26	3.05	3.48	2.58	2.32
7	3.40	2.29	2.96	4.17	3.47	2.38	2.61	3.26	3.05	3.46	2.57	2.32
8	3.40	2.29	2.93	4.14	3.44	2.38	2.60	3.24	3.04	3.43	2.57	2.31
9	3.39	2.28	2.93	4.11	3.43	2.37	2.60	3.24	3.04	3.39	2.56	2.31
10	3.37	2.27	2.92	4.10	3.41	2.37	2.59	3.22	3.04	3.39	2.56	2.31
11	3.36	2.27	2.92	4.09	3.40	2.37	2.58	3.22	3.03	3.38	2.56	2.30
12	3.35	2.27	2.91	4.07	3.39	2.36	2.57	3.20	3.03	3.36	2.55	2.29
13	3.34	2.26	2.91	4.06	3.38	2.36	2.56	3.20	3.02	3.35	2.54	2.28
14	3.33	2.26	2.90	4.04	3.37	2.36	2.56	3.19	3.01	3.32	2.54	2.27
15	3.32	2.26	2.90	4.02	3.36	2.36	2.55	3.18	3.01	3.32	2.53	2.27
16	3.31	2.26	2.90	4.02	3.36	2.36	2.54	3.17	3.00	3.30	2.53	2.26
17	3.31	2.25	2.89	4.02	3.35	2.35	2.53	3.17	3.00	3.28	2.52	2.26
18	3.30	2.25	2.89	4.01	3.34	2.35	2.53	3.16	3.00	3.26	2.51	2.25
19	3.30	2.25	2.87	3.99	3.34	2.35	2.52	3.16	3.00	3.25	2.51	2.25
20	3.29	2.25	2.87	3.98	3.34	2.35	2.52	3.15	2.99	3.25	2.50	2.25
21	3.29	2.24	2.86	3.98	3.33	2.35	2.51	3.14	2.99	3.23	2.50	2.24
22	3.28	2.24	2.86	3.98	3.33	2.34	2.50	3.14	2.99	3.23	2.50	2.23
23	3.28	2.23	2.85	3.97	3.32	2.34	2.50	3.14	2.99	3.20	2.50	2.23
24	3.28	2.23	2.85	3.97	3.32	2.34	2.49	3.12	2.99	3.20	2.50	2.21
25	3.28	2.23	2.85	3.97	3.32	2.34	2.49	3.12	2.98	3.19	2.49	2.21
26	3.28	2.22	2.84	3.94	3.31	2.34	2.49	3.11	2.98	3.19	2.49	2.20
27	3.28	2.22	2.84	3.94	3.31	2.34	2.48	3.10	2.97	3.18	2.49	2.20
28	3.28	2.21	2.84	3.92	3.31	2.34	2.48	3.10	2.96	3.17	2.49	2.20
29	3.28	2.21	2.83	3.92	3.31	2.34	2.48	3.10	2.96	3.17	2.49	2.19
30	3.28	2.21	2.83	3.92	3.31	2.34	2.48	3.10	2.96	3.17	2.49	2.19
%[H^+] gain	91	45	74	129	114	23	58	91	48	151	41	51